F2

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 July 2001 (19.07.2001)

PCT

(10) International Publication Number
WO 01/51490 A1

(51) International Patent Classification[7]: C07D 471/04, 487/04, 239/54, 239/56, 239/58, A61P 29/00, 35/00, A61K 31/505, 31/52 // (C07D 487/04, 239:00, 235:00)

(21) International Application Number: PCT/US01/00981

(22) International Filing Date: 12 January 2001 (12.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/176,373   14 January 2000 (14.01.2000)   US

(71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, 6011 Executive Boulevard, Suite 325, Rockville, MD 20852 (US).

(72) Inventors; and
(75) Inventors/Applicants (for US only): JACOBSON, Kenneth, A. [US/US]; 1111 University Building W, Apartment 504, Silver Spring, MD 20902 (US). MARQUEZ, Victor, E. [US/US]; 220 Doolittle Street, Montgomery Village, MD 20886 (US).

(74) Agent: PILLAI, Xavier; Leydig, Voit & Mayer, Ltd., 700 Thirteenth Street, N.W., Suite 300, Washington, DC 20005 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHANOCARBA CYCLOALKYL NUCLEOSIDE ANALOGUES

(57) Abstract: The present invention provides novel nucleoside and nucleotide derivatives that are useful agonists or antagonists of P1 or P2 receptors. For example, the present invention provides a compound of formula A-M, wherein A is modified adenine or uracil and M is a constrained cycloalkyl group. The adenine or uracil is bonded to the constrained cycloalkyl group. The compounds of the present invention are useful in the treatment or prevention of various diseases including airway diseases (through A_{2B}, A_3, $P2Y_2$ receptors), cancer (through A_3, P2 receptors), cardiac arrhythmias (through A_1 receptors), cardiac ischemia (through A_1, A_3 receptors), epilepsy (through A_1, P2X receptors), and Huntington's Disease (through A_{2A} receptors).

WO 01/51490 A1

METHANOCARBA CYCLOALKYL NUCLEOSIDE ANALOGUES

CROSS-REFERENCE TO A RELATED APPLICATION

This application claims the benefit of U.S. provisional
application No. 60/176,373, filed January 14, 2000, the
disclosure of which is incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

This invention pertains to a novel class of receptor
ligands for P1 and P2 receptors and their therapeutic use.
More specifically, the invention pertains to nucleoside
derivatives in which the sugar moiety is replaced with a
cycloalkyl group that is conformationally constrained by
fusion to a second cycloalkyl group.

BACKGROUND OF THE INVENTION

Purines such as adenosine have been shown to play a wide
array of roles in biological systems. For example,
physiological roles played by adenosine include, inter alia,
modulator of vasodilation and hypotension, muscle relaxant,
central depressant, inhibitor of platelet aggregation,
regulator of energy supply/demand, responder to oxygen
availability, neurotransmitter, and neuromodulator. (Bruns,
Nucleosides & Nucleotides, 10(5), 931-934 (1991)). Because of
its potent actions on many organs and systems, adenosine and
its receptors have been the subject of considerable drug-
development research (Daly, J. Med. Chem., 25, 197 (1982)).
Potential therapeutic applications for agonists include, for

2

instance, the prevention of reperfusion injury after cardiac
ischemia or stroke, and treatment of hypertension and epilepsy
(Jacobson, et al., J. Med. Chem., 35, 407-422 (1992)).
Adenosine itself has recently been approved for the treatment
5   of paroxysmal supra ventricular tachycardia (Pantely, et al.,
Circulation, 82, 1854 (1990)). Adenosine receptor agonists
also find use as anti-arrhythmics, antinociceptives, anti-
lipolytics, cerebroprotectives, and antipsychotics.
        P2 receptors, are present in heart, skeletal, various
10   smooth muscles, prostate, ovary, and brain and have been
implicated in certain aggregation processes associated with
thrombosis and as anti-hypertensive and anti-diabetic agents.
Agonists that bind the P2 receptor induce activation of
phospholipase C, which leads to the generation of inositol
15   phosphates and diacyl glycerol with a subsequent rise in
intracellular calcium concentration and muscle relaxation. P2
receptor antagonists block ADP-promoted aggregation in
platelets and thereby exert an anti-thrombotic effect.
        All P1 and P2 receptor nucleoside ligands suffer from
20   chemical instability that is caused by the labile glycosidic
linkage in the sugar moiety of the nucleoside. However, it
has been found that relatively few ribose modifications are
tolerated by the presently known agonists and antagonists of
P1 and P2 receptors.
25   New compositions are needed that have improved chemical
stability and that do not destroy the activity of such
compounds.
        The invention provides such compositions and methods of
using them in the treatment of disease. These and other
30   advantages of the present invention, as well as additional

inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides novel nucleoside and nucleotide derivatives that are useful agonists or antagonists of P1 or P2 receptors. The invention is premised upon the novel combination of adenine and uracil and their derivatives with a constrained cycloalkyl group, typically a cyclopentyl group. The constraint on the cycloalkyl group is introduced by fusion to a second cycloalkyl group. In the case of cyclopentane, the fusion is typically with cyclopropane. The present compounds retain a surprising binding affinity despite the substitution for the ribose group. Moreover, the absence of the glycosidic bond in the compounds assists in improving the chemical stability of the compounds and aids in overcoming the stabilit problem associated with the glycosidic bond in previously known P1 and P2 receptor ligands.

The compounds of the present invention are useful in the treatment or prevention of various airway diseases (through A_{2B}, A_3, $P2Y_2$ receptors), cancer (through A_3, P2 receptors), cardiac arrhythmias (through A_1 receptors), cardiac ischemia (through A_1, A_3 receptors), epilepsy (through A_1, P2X receptors), Huntington's Disease (through A_{2A} receptors), Immunodeficient disorders (through A_2, A_3 receptors), inflammatory disorders (through A_3, P_2 receptors), neonatal hypoxia (through A_1 receptors), neurodegenerative (through A_1, A_3, P2 receptors), pain (through A_1, A_3, P2X3 receptors), Parkinson's Disease (through A_{2A} receptors), renal failure (through A_1 receptors), schizophrenia (through A_{2A} receptors), sleep disorders (through A_1 receptors), stroke (through A_1, A_3,

4

P2 receptors), thrombosis (through $P2Y_1$, $P2Y_{AC}$ receptors), urinary incontinence (through $P2X_1$ receptors), diabetes (through A_1 receptors), psoriasis (through P2X receptors), septic shock (through P2 receptors), brain trauma (through A_1

5   receptors), glaucoma (through A_3 receptors) and congestive heart failure (through P2 receptors).

    The invention may best be understood with reference to the accompanying drawings and in the following detailed description of the preferred embodiments.

10

## DETAILED DESCRIPTION OF THE INVENTION

    The present invention provides a new class of nucleoside and nucleotide analogs that serve as selective agonists or antagonists for P1 and P2 receptors.

15   Generally, the compounds of the present invention comprise two basic chemical components designated "A" and "M" which are covalently bonded to one another. Component A comprises adenine or uracil, and component M includes a constrained cycloalkyl group. Preferably the adenine and

20   uracil are chemically modified or substituted with moieties that allow the compound to bind to a P1 or P2 receptor. To that end any of a wide variety of chemical groups can be used to modify adenine and uracil. Those groups are well known to those of skill in the receptor art. Preferably, when A is

25   purine or a purine derivative, the linkage between A and M is a chemical bond between the N9 purine nitrogen and the C1 carbon of the cycloalkyl group. Where A is pyrimidine or a pyrimidine derivative, the bond is between N1 pyrimidine nitrogen and the C1 carbon of the cycloalkyl group. The

compounds of the present invention have improved stability and
surprising receptor binding affinity.

While not wishing to be bound to any particular theory,
it is believed that the constrained cycloalkyl group assists
in improving chemical stability and receptor affinity.
Preferably the cycloalkyl groups are capable of adopting a
conformation such that the compound can bind to P1 or P2
receptors. As a result, preferred cycloalkyl groups are those
that tend to form energetically favorable interactions with P1
and P2 receptors and avoid energetically unfavorable ones,
such as unfavorable ionic and/or steric interactions.
Further, the cycloalkyl group is derivatized with a bridging
group. The constraint restricts the cycloalkyl group to
certain conformations that are believed to be beneficial to
binding affinity. The preferred cycloalkyl group is a
cyclopentyl group. With cyclopentyl groups the preferred
method for introducing a conformational constraint is by
derivatizing with a fused cyclopropane bridge. With this
modification the cyclopentane ring is believed to be
constrained to mimic the conformation of a rigid furanose
ring.

Compounds of the present invention include the compounds
shown below in Formulae I and II.
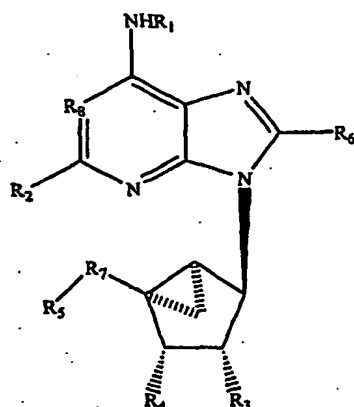
Formula 1
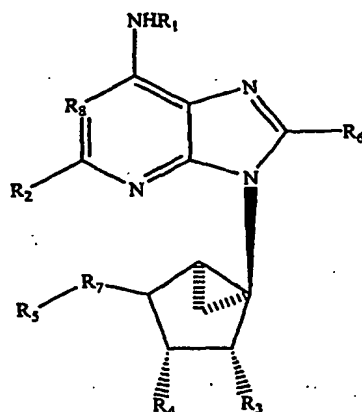
Formula II

Formulae I and II show compounds in which a derivatized
or underivatized adenine base is joined to a constrained

5   cyclopentyl group. For purposes of reference, the carbon atom
of the cyclopentyl group, M, that is joined to adenine, A, is
the C1 carbon and the adenine is joined to M through its N9
nitrogen. In the compounds of Formulae I and II the
constrained cyclopentyl group is derivatized with a fused

10   cyclopropane bridge. In Formula I the cyclopropyl group
bridges carbon atoms C4 and C6. In Formula II the cyclopropyl
group bridges carbon atoms C6 and C1. These distinct bridging
patterns constrain the cyclopentyl group into distinct
conformations, specifically the N- (northern) conformation as

15   in Formula I and the S- (southern) conformation as in Formula
II. These two conformations are thought to mimic the two

biologically active conformations of furanose groups for P1 and P2 receptor binding pockets.

The compounds described by Formulae I and II can be further defined by a variety of suitable modifications to the adenine group. As discussed above, any of a wide variety of chemical groups can be used to form suitable adenine derivatives that comprise the novel compounds of the present invention, provided that the resulting compound is capable of binding to a P1 or P2 receptor. These chemical groups are well known in the art and have been described, for example in U.S. Patent Nos. 5,284,834; 5,498,605; 5,620,676; 5,688,774; and Jacobson and Van Rhee, PURINERGIC APPROACHES IN EXPERIMENTAL THERAPEUTICS, Chapter 6, p. 101 (Jacobson and Jarvis eds., 1997); and Jacobson et al., THE P2 NUCLEOTIDE RECEPTORS, p. 81-107, in THE RECEPTORS (Turner et al. eds. 1998), which are incorporated by reference herein. The combination of the chemically modified adenine and the constrained cycloalkyl group provides a surprising improvement in both chemical stability and binding affinity.

By way of example and not in limitation of the present invention in the compounds of Formulae I and II, R_1 is hydrogen, alkyl, cycloalkyl, alkoxy, cycloalkoxy, aryl, arylalkyl, acyl, sulfonyl, arylsulfonyl, thiazolyl or bicyclic alkyl; R_2 is hydrogen, halo, alkyl, aryl, arylamino, aryloxide, alkynyl, alkenyl, thioether, cyano, alkylthio or arylalkylthio; R_3, R_4, and R_5, are each hydrogen, hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, aryl, acyl, alkylamino, arylamino, phosphoryl, phosphonyl, boronyl, or vanadyl, and they can be the same or different; R_6 is hydrogen, alkyl, alkenyl, alkynyl, or aminoalkyl. R_7 is a methylene,

8

dihalomethyl, carbonyl, or sulfoxide group. R_8 is carbon or
nitrogen. At least one of R_1, R_2, and R_6 is not hydrogen. It
can be appreciated that various combinations of the above
groups are also within the invention provided that they retain

5   agonist or antagonist activity with a P1 or P2 type receptor.

Where an alkyl, alkenyl, alkynyl group is referenced by
itself or as part of another group, the reference is to an
uninterrupted carbon chain consisting of no more than 20
carbon atoms. Aryl and cycloalkyl groups contain no more than

10   8 carbons in the ring.

Reference to alkyl groups is further meant to include
straight or branched chain alkyls, arylalkyl, aminoalkyl,
haloalkyl, alkylthio or arylalkylthio groups. Alkyls
specifically include methyl through dodecyl. Where alkyl

15   groups are present at position R_6 in adenine, it is preferred
that the chain length be no longer than 6 carbons. Arylalkyl
groups include, phenylisopropyl, phenylethyl. Aminoalkyl
groups can be any suitable alkyl group also containing an
amine. Similarly, haloalkyl groups can be any suitable alkyl

20   group that contains a halo substituent, such as bromo, chloro,
flouro, iodo. Alkylthio includes such moieties as thiomethyl,
thiopentyl, thiohexyl, thioheptyl, thiooctyl, thiodecyl,
thioundecyl, ethylthioethyl, or 6-cyanohexylthio groups.
Alkylthio also is meant to include arylalkylthio such as 2-(p-

25   nitrophenyl)ethyl)thio, 2-aminophenylethylthio, 2-(p-
nitrophenyl)ethylthio, or 2-aminophenylethylthio.

Cycloalkyls for example cyclopentyl, cyclohexyl,
hydroxycyclopentyl.

Alkoxys include for example methoxy groups.

9

Cycloalkoxys can include cyclopentoxy.

Aryl moieties can be arylalkyl, arylalkylthio, arylsulfonyl, arylamino, aryloxide, heteroaryl, haloaryl, arylurea, arylcarboxamido, heteroarylamino or sulfoaryl. Benzyl groups are one species of aryl group. In addition, the arylalkyls include R-phenylisopropyl or phenylethyl. Aryloxides can be phenyl, R-phenylisopropyl, phenylethyl, 3,5-dimethoxyphenyl-2-(2-methylphenyl)ethyl and sulfophenyl. Haloaryl can be iodobenzyl among other halogenated aryl groups. Additionally, the heteroaryls include, for example, furans such as tetrahydrofuran.

Acyl groups include carbonyls.

Alkenyl groups are analogous to alkyl groups but include at least one carbon-carbon double bond. When present at the R_6 group of adenine it is preferred that the carbon chain length be from 2 to 6 carbons.

Similarly, alkynyls are analogous to alkenyl groups but contain at least one triple carbon-carbon bond. As with other groups, when present at the R_6 position of adenine it is preferred that they are not longer than 6 carbons.

Phosphoryl groups include diphosphoryl, triphosphoryl, thiophosphoryl, thiodiphosphoryl, thiotriphosphoryl, imidodiphosphate, imidotriphosphate, methylene diphosphate, methylenetriphosphate, halomethylene diphosphate, halomethylene triphosphate, boranophosphate, boranodiphosphate, boranotriphosphate, or phosphorothioate-2-thioether for example.

Thio groups include alkylthio, arylalkylthio, alkenylthio, or arylthios. Alkylthio includes such groups as

thiomethyl, thiopentyl, thiohexyl, thioheptyl, thiooctyl, thiodecyl, thioundecyl, ethylthioethyl, or 6-cyanohexylthio. Alkenylthio includes 5-hexenylthio. Arylthios include 2-(p-nitrophenyl)ethyl)thio, 2-aminophenylethylthio, 2-(p-nitrophenyl)ethylthio, or 2-aminophenylethylthio.

One example of a suitable thiazolyl is (benzothiazolyl)thio-2-propyl.

Examples of bicycloalkyls include s-endonorbornyl, or carbamethylcyclopentane.

Halo groups include such elements as fluoro, bromo, chloro, or iodo.

It will also be appreciated that any group that may be further substituted can be, and still be within the scope of the invention. For example, all of the R_1 groups except hydrogen can be further substituted. By way of illustration, when R_1 is not hydrogen, it can be further modified by substitutions with any of the following chemical substituents including amino, cyano, alkoxyl, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, arylalkyl, acyl, halo, hydroxy, phosphoryl, sulfonyl, sulfonamido, carboxyl, thiohydroxyl, sulfonamido, carboxyl, and carboxamido groups. Similarly, for R_2 - R_{10} all of the groups other than hydrogen can be substituted further. Multiple substitutions are also contemplated.

In a preferred embodiment R_1 can be either methyl, cyclopentyl, cyclohexyl, phenyl, R-phenylisopropyl, benzyl, or phenylethyl; R_2 is chloride; and R_6 can be a C_1-C_6 alkylamino, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl group.

Other compounds of the present invention include the compounds shown below in Formulae III and IV. The Formulae

show compounds in which a derivatized or underivatized uracil
base is joined to a constrained cyclopentyl group.
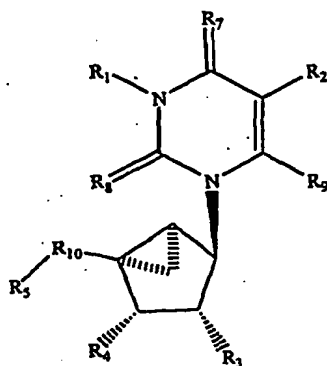
Formula III
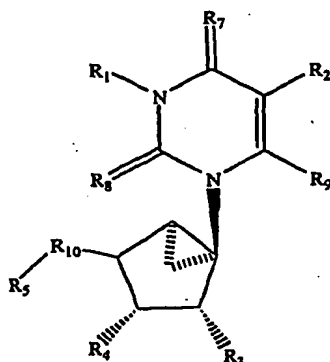
Formula IV

5   The compounds defined by formulae III and IV can be further
     defined by a variety of suitable modifications. For example
     R_1 can be hydrogen, or an alkyl group; R_2 can be hydrogen, C_1-C_6
     alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, or a C_1-C_6 aminoalkyl group;
     R_3, R_4, R_5, can each independently be the same as discussed
10   previously with respect to Formulae 1 and Formulae II. R_6 and
     R_7 are each independently either sulfur or oxygen.

     Certain compounds of the present invention are ligands of
     P2 receptors. A variety of P2 receptors are known in the art
     and the present compounds act at one or more of these, which
15   include for example, P2X and P2Y receptors. These Receptor
     ligands are compounds that bind receptors, preferably in the
     binding pocket. In certain embodiments the compound can be a

P2 receptor agonist. In other embodiments the compound can be a P2 receptor antagonist.

Certain compounds of the present invention are ligands for the P1 receptor. A variety of subclasses of P1 receptors are known and various of present compounds act at one or more these species, which include for example A_1, A_2, and A_3 receptors. Certain compounds act as P1 receptor agonists while others appear to act as antagonists.

The compounds of the present invention are useful in the treatment or prevention of various airway diseases (through A_{2B}, A_3, $P2Y_2$ receptors), cancer (through A_3, P2 receptors), cardiac arrhythmias (through A_1 receptors), cardiac ischemia (through A_1, A_3 receptors), epilepsy (through A_1, P2X receptors), Huntington's Disease (through A_{2A} receptors), Immunodeficient disorders (through A_2, A_3 receptors), inflammatory disorders (through A_3, P_2 receptors), neonatal hypoxia (through A_1 receptors), neurodegenerative (through A_1, A_3, P2 receptors), pain (through A_1, A_3, P2X3 receptors), Parkinson's Disease (through A_{2A} receptors), renal failure (through A_1 receptors), schizophrenia (through A_{2A} receptors), sleep disorders (through A_1 receptors), stroke (through A_1, A_3, P2 receptors), thrombosis (through $P2Y_1$, $P2Y_{AC}$ receptors), urinary incontinence (through $P2X_1$ receptors), diabetes (through A_1 receptors), psoriasis (through P2X receptors), septic shock (through P2 receptors), brain trauma (through A_1 receptors), glaucoma (through A_3 receptors), and congestive heart failure (through P2 receptors).

The present invention is further directed to a pharmaceutical composition comprising a pharmaceutically

13

acceptable carrier and at least one compound selected from the group consisting of the presently described compounds.

The pharmaceutically acceptable excipients described herein, for example, vehicles, adjuvants, carriers or

5   diluents, are well-known to those who are skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one that is chemically inert to the active compounds and one that has no detrimental side effects or toxicity under the conditions of

10   use.

The choice of excipient will be determined in part by the particular compound of the present invention chosen, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable

15   formulations of the pharmaceutical composition of the present invention. The following formulations for oral, aerosol, parenteral, subcutaneous, intravenous, intramuscular, interperitoneal, rectal, and vaginal administration are merely exemplary and are in no way limiting.

20   One skilled in the art will appreciate that suitable methods of utilizing a compound and administering it to a mammal for the treatment of disease states, which would be useful in the method of the present invention, are available. Although more than one route can be used to administer a

25   particular compound, a particular route can provide a more immediate and more effective reaction than another route. Accordingly, the described methods are merely exemplary and are in no way limiting.

The dose administered to an animal, particularly human

30   and other mammals, in accordance with the present invention

should be sufficient to effect the desired response. Such
responses include reversal or prevention of the bad effects of
the disease for which treatment is desired or to elicit the
desired benefit One skilled in the art will recognize that
5 . dosage will depend upon a variety of factors, including the
age, species, condition or disease state, and body weight of
the animal, as well as the source and extent of the disease
condition in the animal. The size of the dose will also be
determined by the route, timing and frequency of
10 administration as well as the existence, nature, and extent of
any adverse side-effects that might accompany the
administration of a particular compound and the desired
physiological effect. It will be appreciated by one of skill
in the art that various conditions or disease states may
15 require prolonged treatment involving multiple
administrations.

Suitable doses and dosage regimens can be determined by
conventional range-finding techniques known to those of
ordinary skill in the art. Generally, treatment is initiated
20 with smaller dosages that are less than the optimum dose of
the compound. Thereafter, the dosage is increased by small
increments until the optimum effect under the circumstances is
reached. The present inventive method typically will involve
the administration of about 0.1 to about 300 mg of one or more
25 of the compounds described above per kg body weight of the
individual.

The following examples further illustrate the present
invention but, of course, should not be construed as in any way
limiting its scope. In the examples, unless otherwise noted,
30 compounds were characterized and resonances assigned by 300

MHz proton nuclear magnetic resonance mass spectroscopy using
a Varian GEMINI-300 FT-NMR spectrometer. Also, unless noted
otherwise, chemical shifts are expressed as ppm downfield from
tetramethylsilane. Synthetic intermediates were characterized
5   by chemical ionization mass spectrometry (NH_3) and adenosine
derivatives by fast atom bombardment mass spectrometry
(positive ions in a noba or m-bullet matrix) on a JEOL SX102
mass spectrometer. Low resolution CI-NH_3 (chemical
ionization) mass spectra were carried out with Finnigan 4600
10 mass spectrometer and high-resolution EI (electron impact)
mass spectrometry with a VG7070F mass spectrometry at 6kV.
Elemental analysis was performed by Atlantic Microlab Inc.
(Norcross, GA). NMR and mass spectra were consistent with the
assigned structure.

15   EXAMPLE 1

In all of the potent adenosine agonists previously
developed, the ribose moiety is present, and consequently,
these agonists are subject to deglycosylation and other
pathways of metabolic degradation in vivo. In order to design
20 non-glycosyl adenosine agonists and thereby increase
biological stability and potential receptor selectivity,
carbocyclic modifications of the ribose moiety have been
introduced. In previous studies of adenosine analogues it was
found that if adenosine derivatives having carbocyclic
25 modifications of the ribose ring (compounds 1-4, below)

bind to adenosine receptors it is only with greatly reduced
affinity.

In the present study we have incorporated a complex
30 carbocyclic modification of ribose for use with adenosine

agonists. This modification, wherein only one isomeric form
retains high affinity and receptor selectivity, is the
"methanocarba" ring. In this modification a fused
cyclopropane ring constrains the accompanying cyclopentane
5   moiety to mimic the conformation of a rigid furanose ring.
The furanose ring of nucleosides and nucleotides in solution
is known to exist in a rapid, dynamic equilibrium between a
range of Northern and opposing Southern conformations as
defined in the pseudorotational cycle. For methanocarba
10   analogues, the bicyclo[3.1.0]hexane ring can constrain the
cyclopentane ring into a N-, 2'-exo envelope pucker, and a *S-*,
3' exo form.
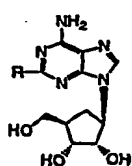
1. R = C₆H₅NH, CGS 23321   2, AMP 579
5b, R = H, aristeromycin
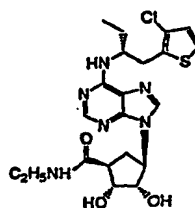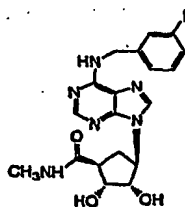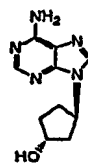
3, MRS 582   4, MDL 201,449

These two extreme forms of ring pucker usually define
15   biologically active conformations. This example shows that
nucleoside binding to P1- (adenosine) receptors, is favored
when the fixed ring-twist conformation is in the N-
conformation.

20   **Chemical Synthesis.**

Nucleosides and synthetic reagents were purchased from Sigma
Chemical Co. (St. Louis, MO) and Aldrich (St. Louis, MO).
2,6-Dichloropurine was obtained from Sigma. m-iodobenzyl
bromide was purchased from Aldrich (St. Louis, MO). 4-(6-
5   Aminopurin-9-yl)-1-hydroxymethyl-bicyclo[3.1.0]hexane-2,3-diol
(1) and compounds 5c and 5d were obtained from Dr. Victor
Marquez. Compounds 7a and 9a were synthesized in our
laboratory.

The synthetic strategy used in this example is shown
10   below. The synthesis of N6-substituted N-methanocarba
adenosine derivatives optimized for interaction with A1 (CP =
cyclopentyl) or A3 (IB = 3-iodobenzyl) receptors. Reagents:
a) DEAD, Ph₃P; b) MEOH, rt; c) BCl₃; d) H2/Pd; e) 3-iodobenzyl
bromide, 50° C, DMF, 2 days; f) NH₄OH, MEOH, 80° C, 3 days.
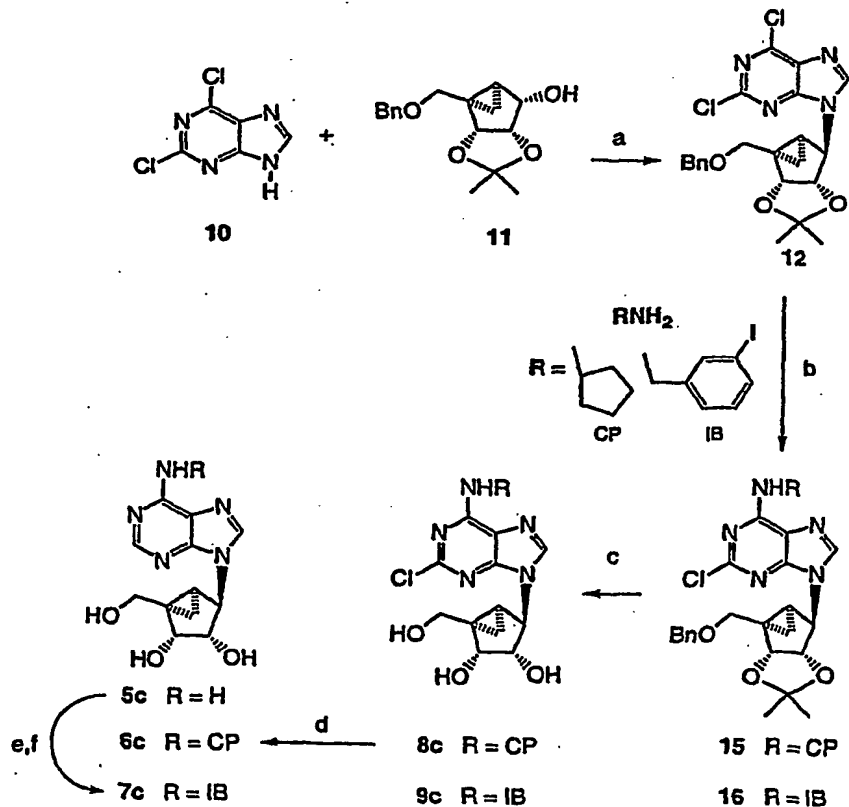
(1'R, 2R, 3'R, 4'R, 1'aR)-2,3-(dihydroxy)-4-(hydroxymethyl)-1-
(6-cyclopentylaminopurine-9-yl)bicyclo(3.1.0)hexane) (6c):

A solution of 8c (4 mg, 0.01 mmol) in methanol (0.5 ml) was
hydrogenated at atmospheric pressure over 10% Pd/C (1 mg) to
furnish the product 6c (83% yield). H'NMR (CD$_3$OD): δ 0.7-0.8
(m, IH, 6'-CHH), 1.46-1.88 (m, 1OH, 6'CHH, 1'aH, 4CH$_2$), 2.01-
2.20 (m, 1H, NCH), 3.34 (d, 1H, J = 9.77 Hz, 5'CHH), 3.88 (d,
1H, J = 6.84 Hz, 3'CH), 4.26 (d, 1H, J = 9.77 Hz, 5'CHH),
4.66-498 (m, 2H, 2'CH, 1'CH), 8.28 (s, 1H, 2CH), 8.5 (s, 1H,
8CH). HRMS(FAB): Cal: 346.1879 Found: 346.1879

(1'R, 2'R, 3'R, 4'R)-2,3-(dihydroxy)-4-(hydroxymethyl)-1-(6-
(3-idobenzylamino)purine-9-yl)cyclopentane (7b):

A mixture of aristeromycin (3.5 mg, 0.013 mmol) and 3-
iodobenzybromide (12 mg, 0.039 mmol) in anhydrous DMF was
heated for 3 days, and solvent was removed under vacuum. The
excess 3-iodobenzylamine was removed from the reaction mixture
by adding ether to the reaction mixture, and stirring was
continued for 5 min. followed by decantation of the
supernatant ether phase. The residue was dried, suspended in
methanol (1 ml) and ammonium hydroxide (0.5 ml), and heated at
80 °C in a closed tube for 1 h. Solvent was removed under
vacuum, and the residue obtained was purified by flash column
chromatography using 7/3 chloroform/methanol to furnish 3.0 mg
(47%) of the product.

H^{1}NMR(CD$_3$OD) δ 1.86-1.96 (m, 1H, 1'CHH), 2.14-2.30 (m, 1H,
1'CHH), 2.38-2.48 (m, 1H, 4'CH), 3.3-3.38 (m, 1H, 5'CHH), 3.67
(d, 1H, J = 6.84 Hz, 5'CHH), 3.96-4.06 (m, 1H, 3'CH), 4.43-
4.48 (m, 1H, 2'CH), 4.73-4.82 (m, 1H, 1'CH), 5.26 (s, 2H,
ArCH$_2$), 7.12 (t, 1H, J = 7.82 Hz, ArH), 7.32 (d, 1H, J =

7.82Hz, ArH), 7.66 (d, 1H, J = 7.82 Hz, ArH), 7.73 (s, 1H, ArH), 8.06 (s, 1H, 2CH). 8.08 (s, 1H, 8CH).

Preparation of 4-[6-(3-iodobenzylamino)-purin-9-yl]-1-hydroxymethyl-bicyclo[3.1.0]hexane-2,3-diol (7c, (N)-

5   Methanocarba-N[6]-(3-iodobenzyl)adenosine) by Dimroth

rarrangement:[1]

To a solution of 4-(6-amino-purin-9-yl)-1-hydroxymethyl-bicyclo[3.1.0]hexane-2,3-diol (5c, 20 mg, 0.0721 mmol) in DMF (0.5 mL) was added m-iodobenzyl bromide (64 mg, 0.216 mmol),

10   and the mixture was stirred at 50 °C for 2 days.   DMF was then removed under a stream of N_2. To the resulting syrup 0.5 mL of acetone and 1 mL of ether were added and the syrup solidified. The solvents were removed by decantation, and again ether was added and removed. The solid was dried and

15   dissolved in 1 mL MEOH. NH_4OH (1.5 mL) was added and the mixture was stirred at 80 °C for 3 days. After cooling down to room temperature, the solvents were removed under reduced pressure and the residue was purified by preparative TLC (silica 60; 1 000 µm; Analtech, Newark, DE; ethyl acetate-i-

20   PrOH-H_2O (8:2:1)) to give 26 mg of the product (7c), yield: 73 %. ^{1}H NMR (CDCl$_3$): δ 0.82 (t, J = 6.0 Hz, 1 H), 1.41 (t, J = 4.8 Hz, 1 H), 1.72 (dd, J = 8.5, 6.0 Hz, 1H), 3.36 (d, J = 10.8 Hz, 1 H), 4.05 (d, J = 6.9 Hz, 1 H), 4.33 (m, 1 H), 4.80-4.88 (m, 3 H), 5.21 (d, J = 6.9 Hz, 1 H), 6.25 (m, br, 1),

25   7.07 (t, J = 7.8 Hz, 1 H), 7.35 (d, J = 7.8 Hz, 1 H), 7.61 (d, J = 7.8 Hz, 1 H), 7.74 (s, 1), 7.93 (s, 1 H), 8.33 (s, 1 H). MS(FAB): m/z 494 (M$^+$+I).

(1'R, 2'R, 3'R, 4'R, 1'aR,)-2,3-(dihydroxy)-4-(hydroxymethyl)-

1-(2-chloro-6-cyclopentylaminopurine-9-

yl)bicyclo(3.1.0)hexane) (8c):

To a solution of 15 (36 mg, 0.076 mmol) in anhydrous

5   dichloromethane was added BCl$_3$ (1M solution in

dichloromethane, 0.23 ml, 0.23 mmol) at 0 °C. The reaction

mixture was warmed to room temperature and stirred for 10 min.

To this mixture was added methanol (1 ml) followed by ammonium

hydroxide (0.5 ml). The mixture was concentrated under

10   vacuum, and the residue obtained was purified by flash column

chromatography using 9/1 chloroform-1/methanol as eluent to

furnish 14 mg of the product 8c (48% yield) as a solid.

H^{1}NMR(CDCl$_3$): δ.o.65-0.9 (m, IH, 6'CHH), 1.1-1.4 (m, 2H, 6'CHH,

1'aH), 1.4-1.9 (m, 8H, 4CH$_2$), 2.0-2.2 (m, 1H, N^{6}CH), 3.34 (d,

15   1H,J = 7.2 Hz, 5'CHH), 3.97 (d, 1H, J = 4.6 Hz, 3'CH), 4.25

(d, 1H, J = 7.2 Hz, 5'CHH), 4.687 (s, 1H, 1'CH), 5.11 (d, 1H,

J 4.6, 2'CH), 7.85 (s, 1H, 8CH). HRMS(FAB): Cal: 380.1489

found: 380.1498

(1'R, 2'R, 3'R, 4'R, 1'aR)-2,3-(dihydroxy)-4-(hydroxymethyl)-

20   1-(2-chloro-6-(3-idobenzylamino)purine-9-

yl)bicyclo(3.1.0)hexane) (9c) was synthesized by the same

method as 8c in 53% yield.

H^{1}NMR(CD$_3$OD): δ 0.70-0.78 (m, 1H, 6'CHH), 1.50-1.63 (m, 2H,

6,,CHH, 1'aH), 3.33 (d, 1H, J = 11.72 Hz, 5'CHH), 3.88 (d, 1H,

25   J = 6.84 Hz, 3'CH), 4.26 (d, 1H, J = 11.72 Hz, 5'CHH), 4.71-

4.83 (m, 2H, 1'CH, 2'CH), 7.1 (t, 1H, J = 7.82Hz, ArH), 7.40

(d, 1H, J = 7.82 Hz, ArH), 7.61 (d, 1H, 7.82 Hz, ArH), 7.78

(s, 1H, ArH), 8.54 (s, 1H, 8CH). HRMS(FAB): Cal: 528.0299

Found: 528.0295

(2R, 3R, 4R, 1'aR, 1S)-2,3-(O-isopropylidine)-4-
(methylenebenzyloxy)-1-(2,6dichloropurine-9-
yl)bicyclo(3.1.0)hexane) (12):

To a solution of triphenyl phosphine (260 mg, 1 mmol) in

5   anhydrous THF (2 ml) was added DEAD (0.16 ml, 1 mmol) dropwise
at 0 °C, and stirring was continued for 20 min. To this
solution was added a solution of 2,6-dichloropurine in THF (4
ml) followed by the addition of 11 (145 mg, 0.5 mmol) in THF
(4 ml). The reaction mixture was warmed to room temperature,

10   and stirring was continued for 6 h. Solvent was evaporated
under vacuum, and the residue obtained was purified by flash
chromatography using 7/3 petroleumether/ethylacetate as eluent
to furnish 141 mg of the product (12) (70% yield) as a gum.
H^1NMR (CDCl$_3$): ∂ 1.0 (m, 1H, 6'C$\underline{H}$H), 1.24 (s, 3H, CH$_3$), 1.27-1.38

15   (m, 1H, 6'CH$\underline{H}$), 1.55 (s, 3H, CH$_3$), 1.62 (dd, 1H, J = 4.88, 9.77
Hz, 1'aH), 3.34 (d, 1H, J = 9.77 Hz, 5'C$\underline{H}$H), 3.97 (d, 1H, J =
9.77 Hz, 5'CH$\underline{H}$), 4.50 (d, 1H, J = 6.84 Hz, 3'CH), 4.57-4.68
(qAB, 2H, J = 12.7 Hz, ArCH$_2$), 5.17 (s, 1H, 1'CH), 5.32 (d,
1H, J = 6.84 Hz, 2'H), 7.27.4 (m, 5H, Ar), 8.63 (s, 1H, 8CH).

20   (2R, 3R, 4R, 1'aR, 1S)-2,3-(O-isopropylidine)-4-
(methylenebenzyloxy)-1-(2-chloro-6-cyclopentylaminopurine-9-
yl)bicyclo(3.1.0)hexane) (15):

To a solution of 12 (42 mg, 0.105 mmol) in methanol (2 ml) was
added cyclopentylamine at room temperature, and stirring was

25   continued for 6 hr for complete reaction. Solvent was removed
under vacuum, and the residue obtained was purified by flash
column chromatography using 7/3 petroleum ether/ethylacetate
as eluent to furnish 45 mg of the product 15 (90% yield) as a
gum.

H^1NMR(CDC1$_3$): δ 0.92-0.96 (m, 1H. 6'C$\underline{H}$H), 1.14-1.01 (m, IH,

6'CH$\underline{H}$), 1.23 (s, 3H, CH$_3$), 1.42-1.81 (m, 9H, 1'aH, 4CH$_2$), 1.54

(s, 3H, CH$_3$), 2.08-2.21 (m, 1H, N^6 C$\underline{H}$), 3.44 (d, 1H, J = 9.76

Hz, 5'C$\underline{H}$H), 3.90 (d, 1H, J = 9.76 Hz, 5'CH$\underline{H}$), 4.51 (d, 1H, J =

6.84 Hz, 3'CH), 4.57-4.67 (qAB, 2H, J = 12.7 Hz, ArCH$_2$), 5.04

(s, 1H, 1'CH), 5.32 (d, 1H, J = 6.84 Hz, 2'CH), 7.2-7.4 (m,

5H, Ar), 8.18 (s, 1H, 8CH).

(1'R, 2'R, 3'R, 4'R, 1'aR)-2,3-(O-isopropylidine)-4-

(methylenebenzyloxy)-1-(2-chloro-6-(3-idobenzylamino)purine-9-

yl)bicyclo(3.1.0)hexane) (16) was synthesized in 70% yield by

the same method as 15, except using 3-iodobenzylamine

hydrochloride and two equivalents of triethylamine.

H^1NMR(CDC1$_3$): δ 0.87-0.91 (m, 1H, 6'C$\underline{H}$H), 1.10-1.29 (m, 1H,

6'CH$\underline{H}$), 1.17 (s, 3H, CH$_3$), 1.42-1.56 (m, 1H, 1'aH), 1.47 (s,

3H, CH$_3$), 3.37 (d, 1H, J = 9.77 Hz, 5'C$\underline{H}$H), 3.84 (d, 1H, J =

9.77 Hz, 5'CH$\underline{H}$), 4.44 (d, 1H, J = 6.84 Hz, 3'CH), 4.50-4.60

(qAB, 2H, J = 11.72 Hz, ArCH$_2$), 4.70 (bs, 1H, NH), 4.98 (s,

1H, 1'CH), 5.24 (d, 1H, J = 6.84 Hz, 2'CH), 7.0 (t, 1H, J =

7.82 Hz, ArH), 7.2-7.34 (m, 6H, ArH), 7.55 (d, 1H, J = 7.82,

ArH), 7.65 (s, 1H, ArH), 8.08 (s, 1H, 8CH).

Pharmacological Analyses.

Materials

F-12 (Ham's) medium, fetal bovine serum (FBS) and

penicillin/streptomycin were from Gibco BRL (Gaithersburg,

MD). [^{125}I]AB-MECA (1000 Ci/mmol) and [^{35}S]guanosine 5'-(γ-

thio)triphosphate (1000-1500 Ci/mmol) were from DuPont NEN

(Boston, MA). Adenosine deaminase (ADA) was from Boehringer

Mannheim (Indianapolis, IN). All other materials were from

23

standard local sources and of the highest grade commercially
available.

Cell culture and membrane preparation

5 CHO cells stably transfected with either human A_1 or A_3
receptors (gift of Dr. Gary Stiles and Dr. Mark Olah, Duke
University Medical Center) were cultured as monolayers in
medium supplemented with 10% fetal bovine serum. Cells were
washed twice with 10 ml of ice-cold phosphate buffered saline,
10 lysed in lysis buffer (10 mM Tris.HCl buffer, pH 7.4,
containing 2 mM $MgCl_2$ and 0.5 mM EDTA), and homogenized in a
Polytron homogenizer in the presence of 0.2 U/ml adenosine
deaminase. The crude membranes were prepared by centrifuging
the homogenate at 1000 x g for 10 min followed by
15 centrifugation of the supernatant at 40,000 x g for 15 min.
The pellet was washed once with the lysis buffer and
recentrifuged at 40,000 x g for 15 min. The final pellets
were resuspended in 50 mM Tris.HCl buffer, pH 7.4, containing
10 mM $MgCl_2$ and 0.1 mM EDTA and stored at -70 °C.
20

Radioreceptor Binding
Determination of binding to adenosine A_1, A_{2A} and A_{2B} receptors
was carried out as reported. Determination of A_3 adenosine
receptor binding was carried out using $[^{125}I]AB$-MECA. Briefly,
25 aliquots of crude transfected CHO cell membranes
(approximately 40 μg protein/tube) were incubated with 0.5 nM
$[^{125}I]AB$-MECA, 10 mM $MgCl_2$, 2 units/ml adenosine deaminase, 50
mM Tris.HCl (pH 7.4) at 37 °C for 60 min. The total volume of
the reaction mixture was 125 μl. Bound and free ligands were
30 separated by rapid filtration of the reaction mixture through

24

Whatman GF/B glass filters. The filters were immediately washed with two 5 ml-portions of ice-cold 50 mM Tris.HCI buffer (pH 7.4). The radioactivity bound to the filters was determined in a Beckman gamma counter. Specific binding was

5   defined as the amount of the radioligand bound in the absence of competing ligand minus the amount of that bound in the presence of 100 μM NECA. Ki-values were calculated using the K_d for $[^{125}I]$AB-MECA binding of 0.56 nM.

10   **Determination of $[3'S]$GTPγS Binding**

$[^{35}S]$GTPγS binding was determined by the method of Lorenzen et al. The incubation mixture contained in a total volume of 125 μl, 50 mM Tris.HCl (pH 7.4), 1 mM EDTA, 10 mM $MgCl_2$, 10 μM guanosine 5'-diphosphate, 1 mM dithiothreitol, 100 mM NaCl,

15   0.2 units/ml adenosine deaminase, 0. 16 nM $[^{35}S]$ GTPγS (about 50,000 cpm) and 0.5% BSA. The CHO cell membranes expressing A_1 or A_3 receptors were preincubated with the above-mentioned assay mixture at 37 °C for 1 h and further incubated for 1 hr after the addition of $[^{35}S]$GTPγS. Incubations were terminated

20   by rapid filtration of the samples through glass fiber filters (Whatman GF/B), followed by two 5 ml washes of the same buffer. After transferring the filters into a vial containing 3 ml of scintillation cocktail, the radioactivity was determined in a scintillation counter.

25

Data analysis. Analyses of saturation binding assays and concentration-response curves were carried out using the GraphPad Prism (GraphPad Software Inc., San Diego, CA). Comparisons between groups were carried out using the unpaired

30   Student's test.

25

RESULTS

Chemical synthesis

The methanocarbocyclic 2'-deoxyadenosine analogues, shown

5   below in Table 1, in which a fused cyclopropane ring

constrains the cyclopentane ring into a rigid envelope

configuration of either a *N-* or *S-* conformation, were

synthesized in a manner similar as shown above.   The *N-*

methanocarba analogues of various N^6-substituted adenosine

10   derivatives, including cyclopentyl and iodobenzyl, in which

the parent compounds are potent and selective agonists at

either A_1 or A_3 receptors, respectively, were prepared. 2,6-

Dichloropurine, **10**, was condensed with the cyclopentyl

derivative, **11**, using the Mitsunobu reaction, followed by

15   substitution at the 6-position and deprotection to give **8c** or

9e. The 2-chloro substitution of compound **8c** was removed by

catalytic reduction to give **6c**. This allowed the

incorporation in the *N-*configuration series of the 2-chloro

modification of adenine, which was of interest for its effect

20   on adenosine receptor affinity. An N^6-(3-iodobenzyl) group

could also be introduced in either aristeromycin, **5b**, or *N-*

methanocarba-adenosine, **5c**, by the Dimroth rearrangement, to

give **7b** and **7c**.

25   **Biological activity**

A pair of methanocarba analogues of adenosine, **5c** and **5d**,

corresponding to *N-* and *S-* conformations of ribose, were

tested in binding assays, the results of which are shown in

Table 1 below, at four subtypes of adenosine receptors. The

30   more synthetically challenging S-isomer (5d) was available

only as the racemate and therefore was tested as such. At rat
A1, rat A2A, and human A3 subtypes, the N-analogue proved to
be of much higher affinity than the S-analogue. At the human
A2B receptor, binding was carried out using [3H]ZM 241,385,

5   however the affinity was too weak to establish selectivity for
a specific isomer. Affinity of N-methanocarba-adenosine, 5c,
vs. adenosine, 5a, was particularly enhanced at the A3
receptor subtype, for which the ratio of affinities of N-to S-
analogues was 150-fold. Although a poor substrate for

10   adenosine deaminase (ADA), the binding curve for 5c was
shifted in the presence of ADA, therefore the affinity values
for 5c and 5d obtained in the absence of ADA are entered in
Table 1, below. The South confomer, 5d, is even a worse
substrate of ADA (100-fold less) which explains why the curves

15   in the presence and absence of ADA for 5d are virtually the
same. Aristeromycin, 5b, bound weakly to adenosine receptors,
with slight selectivity for the A_{2A} subtype. Compound 5c was
more potent than aristeromycin, 5b, in binding to A1 (4-fold)
and A3 (4500-fold) adenosine receptors.

20   Compounds 6c and 8c are patterned after A1 receptor-
selective agonists, while compounds 7c̄ and 9c are patterned
after A3 receptor-selective agonists. Compounds 6 and 7 are
unsubstituted at the 2-position, while compounds 8 and 9
contain the potency enhancing 2-chloro substituent. The N6-

25   cyclopentyl N-methanocarba derivative, 6c, based on CPA, 6a,
maintained high selectivity for A1 receptors, although the
affinity of 6c at rat A1 receptors was 3-fold less than for
6a. In one series it was possible to compare ribose,
cyclopentyl, and N-methanocarba derivatives having the same

30   N6-substitution. The N6-(3-iodobenzyl) derivative, 7c, based
on a 5'-hydroxy analogue, 7a, of IB-MECA, with a Ki value of

27

4.1 nM was 2.3-fold more potent at A3 receptors than the
ribose-containing parent. Thus, the selectivity of 7c for
human A3 versus rat AI receptors was 17-fold. The
aristeromycin analogue, 7b, was relatively weak in binding to
5   adenosine receptors.

Among 2-chloro-substituted derivatives, the N-
methanocarba analogue, 8c, was less potent at Al and A2A
receptors than its parent 2-chloro-N6-cyclopentyladenosine,
8a, and roughly equipotent at A3 receptors. Thus, 8c was 53-
10 fold selective in binding to rat Al vs. human A3 receptors.
The N-methanocarba analogue, 9c, of 2-chloro-N6-
(3iodobenzyl)adenosine, 9a, had Ki values (nM) of 141, 732,
and 2.2 at Al, A2A, and A3 receptors, respectively. Thus, the
2-chloro group slightly enhanced affinity at A3 receptors,
15 while reducing affinity at Al receptors.

The receptor binding affinity upon replacement of ribose
with the N-methanocarba moiety was best preserved for the A3
subtype, at which differences were small. At Al receptors the
loss of affinity for structures 6 - 9 was between 3- and 8-
20 fold. At A2A receptors the loss of affinity was between 6-
and 34-fold.

The agonist-induced stimulation of binding of guanine
nucleotides to activated G-proteins has been used as a
functional assay for a variety of receptors, including
25 adenosine receptors. Binding of [^{35}S]GTP-γ-S was studied in
membranes prepared from CHO cells stably expressing human Al
or A3 receptors (Table 2). The non-selective adenosine
agonist NECA (5'-N-ethyluronamidoadenosine) caused a
concentration-dependent increase in the level of the guanine
30 nucleotide bound. Compound 6c was highly selective and a full
agonist at human Al but not rat Al receptors. Both 7c and 9c

stimulated the binding of [^{35}S]GTP-γ-S, however the maximal stimulation was significantly less than that produced by either NECA or N6(3-iodobenzyl)adenosine, 7a, both being full A3 agonists. Compounds 7c and 9c resulted in relative

5   stimulation of [^{35}S]GTP-γ-S binding of only 45% and 22%, respectively, indicating that the efficacy of the N-methanocarba analogue at A3 receptors was further reduced upon 2-chloro modification. The potency of compounds 7c and 9c, indicated by the EC50 values in this functional assay, was

10   greater than the potencies of either NECA or compound 7a (Table 2). Thus, the N-methanocarba N6-(3-iodobenzyl) analogues appear to be highly potent and selective partial agonists at human A3 receptors.

Table I

Affinities of Adenosine Derivatives[1]

1b-4b R''=OH
1c-4c R''=H

1a-4a
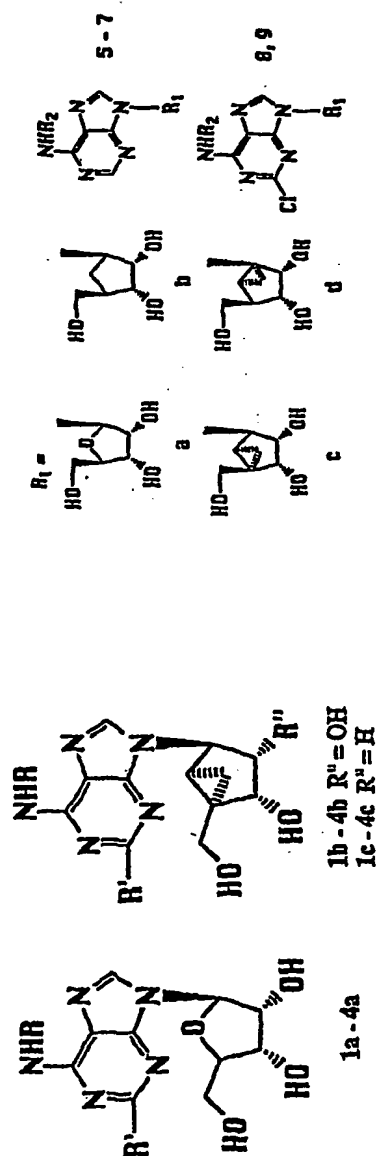
Compound	R'	R	K_i (nM) or % displacement		
			RA1[a]	RA2A[b]	hA3[c]
1a	H	cyclopentyl	0.59	462	274±20
CPA					240 (r)
1B 1781	H	cyclopentyl	5.06±0.51	6800±1800	170±51
1c 1783	H	cyclopentyl	5110±790	15% at 10 µM	
2a IBO-ADO,541	H	3-iodobenzyl	20.0±8.5	17.5±0.5	9.5±1.4 (r)
2b	H	3-iodobenzyl	69.2±9.8	601±236	4.13±1.76

Compound	R_2	R_1	rA_1[a]	rA_{2A}[b]	hA_{2B}[b]	hA_3[b]	A_1/A_3
1743, 3a CCPA	Cl	cyclopentyl	0.6	950		237 (r)	
1761, 3b	Cl	cyclopentyl	8.76±0.81	3390±520		466±58	
1782, 3c	Cl	cyclopentyl	3600±780	45±5% at 100 μM			
542, 4a	Cl	3-iodobenzyl	18.5±4.7	38.5±2.0		1.41±0.17 (r)	
1760, 4b	Cl	3-iodobenzyl	141±22	732±207		2.24±1.45	
1784, 4c	Cl	3-iodobenzyl	8730±370	25,400±3800			
5a	H		Estd. 10[d]	estd. 30[d]	<10% at 100 μM	estd. 1000 (r)[d,e]	100
5b	H		6260±730	2150±950	47,300±10,600	20,000±7900 (r)[e]	0.31
5c	H		1680±80	22,500±100 (h)[e,f]	35±2% at 50 μM[f]	404±70[f]	4.2

5d (racemic)	H	15% at 100µM	>100,000 (h)[e,f]	20±4% at 50 µM[f]	62,500±2900[f]	>1
6a	CP	1.50±0.51	857±163	21,200±4300	274±20, 240 (r)[e]	0.0055
6c	CP	5.06±0.51	6800±1800	139k±19k	170±51	0.030
7a	IB	20.0±8.5	17.5±0.5	3570±100	9.5±1.4 (r)[e]	2.1
7b	IB	25,900±1600	<10% 100µM	n.d.	1960±370	13
7c	IB	69.2±9.8	601±236	12,100±1300	4.13±1.76	17
8a	CP	1.33±0.19	605±154	20,400±1200	237 (r)[e]	0.0056
8c	CP	8.76±0.81	3390±520	27±7% at 100 µM	466±58	0.019
9a	IB	18.5±4.7	38.5±2.0	5010±1400	1.41±0.17 (r)[e]	13
9c	IB	141±22	732±207	41,000±700	2.24±1.45	63

[1] (a) simple carbocyclic, (b) and methanocarba-adenosine (N)-conformation; (c) and S-conformation, (d) derivatives in radioligand binding assays at rat A_1,[a] rat A_{2A},[b] human A_{2B},[b] and human A_3 receptors,[c] unless noted.[e]

32

TABLE II

Effect of ligands to stimulate [^{35}S] GTPγS binding to membranes of cells expressing the cloned hA$_1$AR or hA$_3$AR or in rat cerebral cortical membranes containing the A$_1$AR

Ligand	cloned hA$_1$AR EC$_{50}$ (nM)[a]	% Maximal Stimulation[c]	rA$_1$AR EC$_{50}$ (nM)[a]	% Maximal Stimulation[c]	cloned hA$_3$AR EC$_{50}$ (nM)[a]	% Maximal Stimulation[c]
NECA	n.d.		n.d.		155±15	100
6a	4.15 ± 0.90	100	20.3 ± 13.1	100	7980 ± 60	100
6c	21.5 ± 2.3	102 ± 1	100 ± 17	75 ± 6	>10,000	14±2% at 10 µM
7a	43.1 ± 10.4	91 ± 1	340 ± 98	95 ± 4	5.16 ± 0.71	100
7b	>10,000	5±2% at 10 µM	n.d.		>10,000	15±5% at 10 µM
7c	218 ± 18	86 ± 2	940 ± 114	55 ± 5	0.70 ± 0.16	45.3 ± 6.8
8c	31.2 ± 3.3	97 ± 1	145 ± 35	96 ± 2	n.d.	
9c	142 ± 24	91 ± 1	684 ± 75	48 ± 3	0.67 ± 0.19	22.0 ± 2.8

a EC$_{50}$ for stimulation of basal [^{35}S]GTP-γ-S binding by agonists in membranes from transfected CHO cells (±S.E.M.), n = 3.

n.d. not determined.

5

Discussion

Nearly all of the thousands of known adenosine agonists are derivatives of adenosine. Although molecular modeling of adenosine agonists has been carried out, there has been no

10 direct evidence from this for a conformational preference of the ribose ring in the receptor binding site. In the present study, methanocarba-adenosine analogues have defined the role of sugar puckering in stabilizing the active receptor-bound conformation. The S-methanocarba analogue of adenosine, 5d,

15 was only weakly active, presumably because of a disfavored conformation that decreases receptor binding. In contrast, the methanocarba analogues constrained in the N-conformation, e.g. 5c - 9c, displayed high receptor affinity, particularly at the A3 receptor. In binding assays at A1, A2A, and A3

20 receptors, N-methanocarba-adenosine proved to be of higher

affinity than the S-analogue, with an $N:S$-affinity ratio of
150 at the human A3 receptor. Thus, the biological potency
and efficacy of this series of nucleosides appears to be
highly dependent on ring puckering, which in turn would
5 influence the orientation of the hydroxyl groups within the
receptor binding site.

The structure activity relationship (SAR) of adenosine
agonists indicates that the ribose ring oxygen may be
substituted with carbon, as in 5b and 7b, however much
10 affinity is lost. As demonstrated with the aristeromycin
derivative, 7b, simple carbocyclic substitution of the ribose
moiety of otherwise potent, N6-subsituted adenosine agonists
greatly diminishes affinity, even in comparison to
aristeromycin, 5b.

15 In comparison to the ribose analogues, the N-methanocarba
N6-subsituted adenosine agonists were of comparable affinity
at A3 receptors, but less potent at A1, A2A, and A2B
receptors. The N-methanocarba N6-cyclopentyl derivatives were
A1 receptor-selective and maintained high efficacy at human
20 recombinant but not rat brain A1 receptors, as indicated by
stimulation of binding of [^{35}S]GTPγS. This may be related to
either species differences or heterogeneity of G proteins,
since the degree of agonist efficacy of a given compound may
be highly dependent on the receptor-associated G protein. N-
25 Methanocarba N6-(3-iodobenzyl)adenosine and the 2-chloro
derivative had Ki values of 4.1 and 2.2 nM at A3 receptors,
respectively, and were selective partial agonists. As for the
ribose parents, additional 2-chloro substitution was favorable
for receptor selectivity. However, unlike the ribose forms,
30 efficacy was reduced in N6-(3-iodobenzyl) analogues, such that
partial A3 receptor agonists 7c and 9c were produced.

Partial agonists are possibly more desirable than full
agonists as therapeutic agents due to potentially reduced side
effects in the former. Partial agonists may display *in vivo*
specificity for sites at which spare receptors are present,
5   and the drug would therefore behave with apparent "full"
efficacy. Thus, for compounds 7c and 9c, partial agonism
combined with unprecedented functional potency at A3 receptors
(<1 nM) may give rise to tissue selectivity.

Thus, at least three of the four adenosine receptors
10   favor the N-conformation. For another member of the GPCR
superfamily, the P2Y1 receptor, we recently reported that the
ribose N-conformation of adenine nucleotides also appears to
be preferred at the receptor binding site. Thus, the P1 and
at least one of the P2 purinoceptors share the preference for
15   the N-conformation. This may suggest a common motif of
binding of nucleoside moieties among these GPCRS. The
insights of this conformational preference may be utilized in
simulated docking of adenosine agonists in a putative receptor
binding site and to design even more potent and selective
20   agents.

At the binding site of ADA, the N-isomer is also
preferred, although the carbocyclic adenosine analogues are
relatively poor substrates (relative rates of deamination are:
5a, 100; 5b, 0.99; 5c, 0.58; 5d, 0.010, N6-substituted
25   analogues, such as 6c - 9c, would not be expected to be
substrates for ADA. Other enzymes, such as HIV reverse
transcriptase and Herpes thymidine kinase (HSV-1 TK) are also
able to discriminate between the two antipodal conformations
of restricted methanocarba thymidine analogues.

30   In conclusion, we have found that the introduction of a
methano-carbocyclic modification of the ribose ring of purine

agonists represents a general approach for the enhancement of
pharmacodynamic and because of the absence of the glycosyl
bond, potentially of pharmacokinetic properties. This
approach could therefore be applied to the development of
5   cardioprotective, cerebroprotective, and anti-inflammatory
agents.

EXAMPLE 2

Introduction

10   P2 receptors, which are activated by purine and/or
pyrimidine nucleotides, consist of two families: G protein-
coupled receptors termed P2Y, of which 5 mammalian subtypes
have been cloned, and ligand-gated cation channels termed P2X,
of which 7 mammalian subtypes have been cloned. The $P2Y_1$
15   receptor, which is present in the heart, skeletal and various
smooth muscles, prostate, ovary, and brain, was the first P2
subtype to be cloned. The nomenclature of P2 receptors and
their various ligand specificities is well established.

        Nucleotide agonists binding at $P2Y_1$ receptors induce
20   activation of phospholipase C (PLC), which generates inositol
phosphates and diacylglycerol from phosphatidyl inositol-
(4,5)-*bis*phosphate, leading to a rise in intracellular
calcium. A $P2Y_1$ receptor antagonist may have potential as an
anti-thrombotic agent, while a selective $P2Y_1$ receptor agonist
25   may have potential as an anti-hypertensive or anti-diabetic
agent.

        Recently, progress in the synthesis of selective P2
receptor antagonists has occurred. Adenosine 3',5'- and
2',5'-bisphosphates were recently shown to be selective
30   antagonists or partial agonists at $P2Y_1$ receptors, and other

36

classes of P2 antagonists include pyridoxal phosphate derivatives, isoquinolines, large aromatic sulfonates related to the trypanocidal drug suramin and various dyestuffs, and 2',3'-nitrophenylnucleotide derivatives. Synthesis of analogues of adenosine *bis*phosphates has resulted in N6-methyl-2'-deoxyadenosine-3',5'-bisphosphate (1a, MRS 2179), a competitive antagonist at human and turkey $P2Y_1$ receptors, with a KB value of approximately 100 Nm. The presence of an N^6-methyl group and the absence of a 2'-hydroxyl group both enhanced affinity and decreased agonist efficacy, thus resulting in a pure antagonist at both turkey and human $P2Y_1$ receptor. The corresponding 2-Cl analogue (1b, MRS 2216) was slightly more potent than 1a as an antagonist at turkey $P2Y_1$ receptors, with an IC_{50} value of 0.22 μM in blocking the effects of 10 nm 2-methylthioadenosine-5'diphosphate (2-MeSADP). MRS2179 (compound 1a) was inactive at $P2Y_2$, $P2Y_4$, and $P2Y_6$ subtypes, at the adenylyl cyclase-linked P2Y receptor in C6 glioma cells and at a novel avian P2Y receptor that inhibits adenylyl cyclase. However, the selectivity of this series of nucleotides for the $P2Y_1$ receptor is not absolute, since 1a also displayed considerable activity at $P2X_1$ receptors (EC_{50} 1.2 μM), but not at $P2Y_{2-4}$ receptors.

In order to move away from the nucleotide structure of 1a and thereby increase biological stability and selectivity for the receptors in the present study, further structural modifications of the ribose moiety have been carried out. We have explored the SAR of these two series and introduced major modifications of the ribose moiety. These modifications include fixing the ring pucker conformation in the carbocyclic

series using a bridging cyclopropane ring, ring enlargement with introduction of a nitrogen atom, and ring contraction.
Results
Chemical synthesis

5   The methanocarbocyclic 2'-deoxyadenosine analogues in which the fused cyclopropane ring fixes the conformation of the carbocyclic nucleoside into a rigid northern or southern envelope conformation, as defined in the pseudoroational cycle, were synthesized as precursors of nucleotides 4 and 5

10 by the general approach of Marquez and coworkers. Again, the N^6-methyl group was introduced by the Dimroth rearrangement, as shown below.
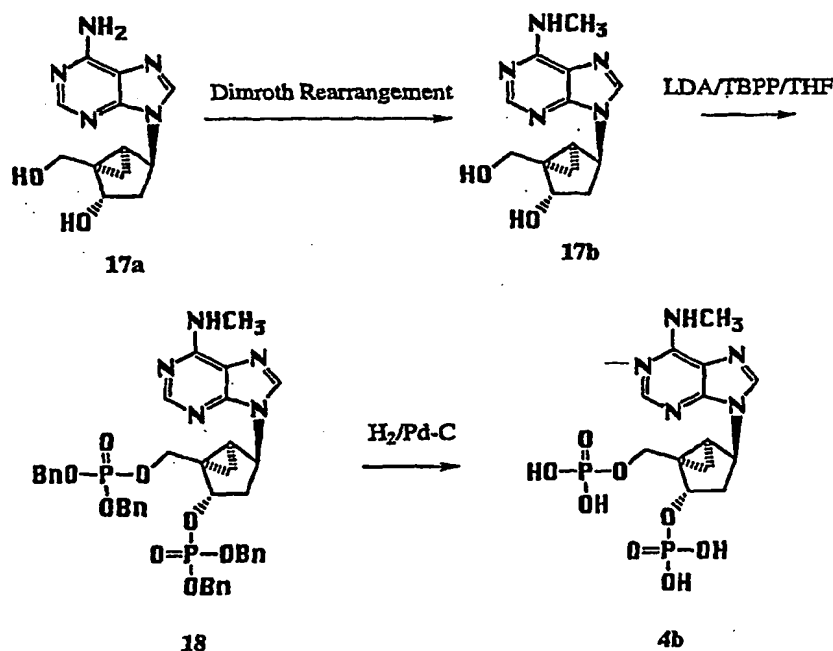
Position adenine modifications were further introduced in the

15 N-configuration series as shown below.
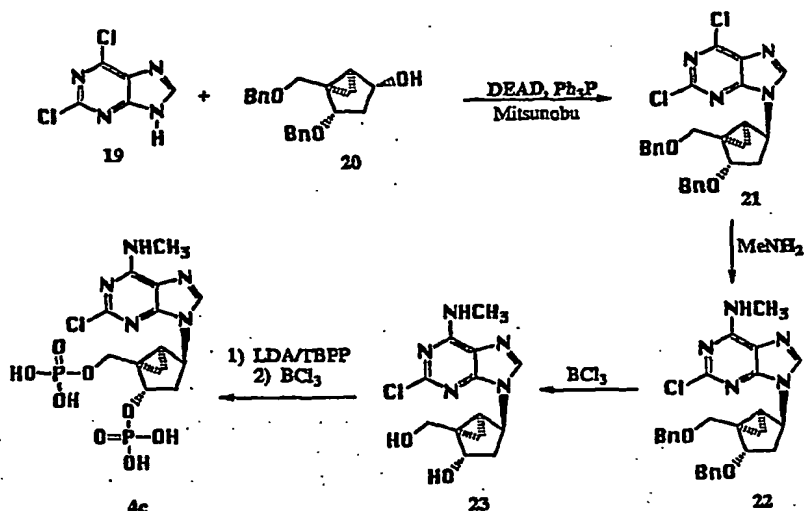
Biological activity

Adenine nucleotides markedly stimulate inositol lipid hydrolysis by phospholipase C in turkey erythrocyte membranes,

5 through activation of a P2Y$_1$ receptor. The agonist used in screening these analogues, 2-MeSADP, has a higher potency than the corresponding triphosphate for stimulation of inositol phosphate accumulation in membranes isolated from [^{3}H]inositol-labeled turkey erythrocytes.

10 The deoxyadenosine *bis*phosphate nucleotide analogues prepared in the present study were tested separately for agonist and antagonist activity in the PLC assay at the P2Y$_1$ receptor in turkey erythrocyte membranes, and the results are reported in Table 3. Concentration-response curves were

15 determined for each compound alone and in combination with 10nM 2-MeSADP.

Marquez and coworkers have introduced the concept of ring-constrained carbocyclic nucleoside analogues, based on cyclopentane rings constrained in the *N*-(Northern) and *S*-

20 (Southern) conformations by fusion with a cyclopropane

(methanocarba) ring. In the presnet studies the series of

ring-constrained N-methanocarba derivatives, the $6-NH_2$

analogues, 4a was a pure agonist of EC_{50} 152 nM and 88-fold more

potent than the corresponding S-isomer, 5, also an agonist.

5. Thus, the ribose ring N-conformation appeared to be favored in

recognition at $P2Y_1$ receptors. The N^6-methy- and 2-chloro-N^6-

methyl-N-methanocarba analogues, 4b and 4c, were antagonists

having IC_{50} values of 276 and 53 nM, respectively.

10 **Molecular modeling.**

To better understand the role of the sugar puckering on

the human $P2Y_1$ agonist and antagonists activities, we carried

out a molecular modeling study of this new generation of

ribose-modified ligands. Such modifications include

15 cyclopentyl rings constrained in the N- and S- conformations

with cyclopropyl (methanocarba) groups, six-membered rings

(morpholino and anhydrohexitol analogues), and cyclobutyl

nucleotides. We have recently developed a model of the human

$P2Y_1$ receptor, using rhodopsin as a template, by adapting a

20 facile method to simulate the reorganization of the native

receptor structure induced by the ligand coordination (*cross-

docking* procedure). Details of the model building are given

in the Experimental Section. We have also reported the

hypothetical molecular basis for recognition by human $P2Y_1$

25 receptors of the natural ligand ATP and the new potent,

competitive antagonist 2'-deoxy-N^6-methyladenosine-3',5'-

*bis*phosphate. Both ATP and 1a are present in the hypothetical

binding site with a N-sugar ring conformation. In the present

work, the sterically constrained N- and S-methanocarba agonist

40

analogues, 4a and 5, respectively, were docked into the putative binding site of our previously reported P2Y$_1$ receptor model. According to their structural similarity, the *cross-docking* procedure demonstrated that the receptor architecture
5 found for binding the ATP and 1a was energetically appropriate also for the binding of both 4a and 5. However, *N*-methanocarba/ P2Y$_1$ complex appeared more stable by approximately 20 kcal/mol than *S*-methanocarba/P2Y$_1$ complex.

In the lowest energy docked complex of *N*-methanocarba agonist
10 in the proposed ligand binding cavity the side chain of Gln307 is within hydrogen bonding distance of the N^6 atom at 1.8Å, and the side chain of Ser314 is positioned at 2.0Å from the N^1 atom and at 3.4Å from the N^6 of the purine ring. As already reported, another three amino acids are important for the
15 coordination of the phosphate groups in the antagonist: Arg128, Lys280 and Arg310. Lys280 may interact directly with both 3'-5'-phosphates (1.7Å, O3'and 1.7Å, O5'), whereas Arg128 and Arg310 are within ionic coupling range to both the O2 and O3 atoms of the 5'-phosphate. In molecular modeling studies
20 poor superimposition (*rms*=1.447) between the *N*- and *S*-methanocarba agonist analogues has been found inside the receptor binding domain. In particular, the adenine moiety and 5' phosphate of the *S*-methanocarba derivative are shifted out position relative to with the *N*-methanocarba isomer,
25 decreasing the stability of the *S*-methanocarba/PSY$_1$ complex. This fact might be correlated with the difference of their biological activity as seen in Table 4 below.

Using the information that a common binding site could be hypothesized among these deoxyadenosine bisphosphate
30 analogues, a superimposition analysis of the energy-minimized

of the more potent antagonists has been performed. In this

analysis we have used 1a as a reference compound, and we have

defined three matching pairs of atoms, corresponding to N^1

atom of the purine ring and the P atoms of both 3' and 5'

5   phosphate groups, to carry out the superimposition analysis.

As reported in Table 4, acceptable RMS values have been

obtained for all the antagonists compared with the 1a

structure. As shown in Fig 4A, this superimposition study

suggested that the two phosphate groups may occupy a common

10 receptor regions, and a general pharmacophore model for

bisphosphate antagonists binding to the human PSY_1 receptor

can be extrapolated.

Discussion

15   In conclusion the present study has identified new

pharmacological probes of PSY_1 receptors, including full

agonists, partial agonists, and antagonists. The SAR of 1a

indicates that the ribose ring oxygen may be readily

substituted with carbon. Furthermore, analogues of

20 constrained conformation, e.g. the methanocarba analogues,

display enhanced receptor affinity. Additional 2-chloro and

N^6-methyl substitution is favorable for affinity at PSY_1

receptors, and nearly pure antagonism is maintained provided

that the N^6-methyl group is present.

25   Thus, the biological potency and efficacy of this series

of bisphosphates appears to be highly dependent on subtle

conformational factors, which would influence the orientation

of the phosphate groups within the receptor binding site.

         The sugar moiety of nucleosides and nucleotides in

30 solution is known to exist in a rapid, dynamic equilibrium

42

between extreme 2—*exo*/3'-*endo* (*N-*) and 2'-*endo*/3'-*exo* (*S-*)
conformations as defined in the pseudorotational cycle. While
the energy gap between *N-* and *S-*conformation is in the
neighborhood of 4kcal/mol, such a disparity can explain the
difference between micromolar and nanomolar binding
affinities. Using a molecular modeling approach, we have
analyzed the sugar conformational requirements for a new class
of bisphosphate ligands binding to the human PSY_1 receptor.
As experimentally shown, the ribose ring Northern conformation
appeared to be favored in recognition at human PSY_1 receptor
(see Table 4). We have found new support to our recently
presented hypothesis in which three important recognition
regions are present in the bisphosphate molecular structures;
The N^1 atom of the purine ring and the P atoms of both 3' and
5' phosphate groups. The *N-*conformation seems to be essential
to maximize the electrostatic interactions between the
negatively charged phosphates and the positively charged amino
acids present in the receptor binding cleft, as well Arg128,
Lys280, and Arg310.

Interestingly, the electrostatic contacts also appear to
be crucial for the recognition of bisphosphate antagonists.
Using superimposition analysis, a general pharmacophore model
for the bisphosphate antagonists binding to the PSY_1 receptor
has been proposed. According to the pharmacophore map,
recognition of the bisphosphates antagonists at a common
region inside the receptor binding site and, consequently, a
common electrostatic potential profile is possible. As well
for the agonists, the Northern conformation seems to be
essential to maximize the electrostatic interactions between
the negatively charged phosphates and the positively charged

43

amino acids presents in the receptor binding cleft. As we
predicted using the previously reported PSY_1 receptor model,
sugar moiety does not seen to be crucial for the ligand
recognition process.

5   As already described, the simple addition of the N^6-
methyl group in several cases converted pure agonists to
antagonists. From a pharmacological point of view, this is
really a unique situation. With the addition of the N^6-methyl
group it is not possible to have a double hydrogen-bonding
10   interaction and, consequently, the activation pathway is
blocked. However, for all the N^6-methyl antagonists the
possibility to participate in at least one of the two possible
hydrogen bonds appears to be very important for the increase
in affinity at the PSY_1 receptor.

15

Chemical Synthesis

          Nucleosides and synthetic reagents were purchased from
Sigma Chemical Co. (St. Louis, MO) and Aldrich (St. Louis,
MO). 6-Chloro-2'-deoxypurine riboside was obtained from
20   Sigma. Several 2'-deoxynucleosides, including an
anhydrohexitol-adenine nucleoside and 2'-deoxyaristeromycin
were also synthesized.

          Purity of compounds was checked using a Hewlett-Packard
1090 HPLC apparatus equipped with an SMT OD-5-60 RP-C18
25   analytical column (250 x 4.6 mm; Separation Methods
Technologies, Inc., Newark, DE) in two solvent systems.
System A: Linear gradient solvent system: 0.1 M $TEAA/CH_3CN$
from 95/5 to 40/60 in 20 min and the flow rate was of 1
mL/min. System B: linear gradient solvent system: 5 mM
30    $TBAP/CH_3CN$ from 80/20 to 40/60 in 20 min and the flow rate was

of 1 mL/min. Peaks were detected by UV absorption using a
diode array detector. All derivatives showed more than 95%
purity in the HPLC systems.

Purification of most of the nucleotide analogues for
5 biological testing was carried out on DEAE-A25 Sephadex
columns as described above. However, compounds 7b and 8a – c
required HPLC purification (system a, semi-preparative C18
column) of the reaction mixtures.

General procedure of phosphorylation.

10 **Method A:** The nucleoside (0.1 mmol) and Proton Sponge® (107
mg, 0.5 mmol) were dried for several h in high vacuum at room
temperature and then suspended in 2 mL of trimethyl phosphate.
Phosphorous oxychloride (Aldrich, 37 µL, 0.4 mmol) was added,
and the mixture was stirred for 1 h at 0° C. The reaction was
15 monitored by analytical HPLC (eluting with a gradient
consisting of buffer: CH_3CN in the ratio 95 : 5 to 40 : 60, in
which the buffer was 0.1 M triethylammonium acetate (TEAA);
elution time was 20 min; flow rate was 1 mL/min; column was
SMT OD –5-60 RP-C18; detector was by UV in the E_{max} range of
20 260-300 nm). The reaction was quenched by adding 2 mL of
triethylammonium bicarbonate buffer and 3 mL of water. The
mixture was subsequently frozen and lyophilized. Purification
was performed on an ion-exchange column packed with Sephadex-
DEAE A-25 resin, a linear gradient (0.01 to 0.5 M) of 0.5 M
25 ammonium bicarbonate was applied as the mobile phase, and UV
and HPLC were used to monitor the elution. All nucleotide
bisphosphates were collected, frozen and lyophilized as the
ammonium salts. All synthesized compounds gave correct
molecular mass (high resolution FAB) and showed more than 95%
30 purity (HPLC, retention times are reported in Table 4).

Method B: Nucleoside (0.1 mmol) dried for several h in high vacuum at room temperature was dissolved in 2 mL of dry THF. Lithium diisopropylamide solution (Aldrich, 2.0 M in THF, 0.4 mmol) was added slowly at -78 °C. After 15 min tetrabenzyl
5 pyrophosphate (Aldrich, 0.4 mmol) was added and the mixture was stirred for 30-60 min at -78°C. The reaction mixture was warmed to 0°C - rt and stirred for an addition period ranging from 2h to 24 h. Chromatographic purification (pTLC, $CHCl_3:CH_3OH$ (10:1) gave the tetrabenzyl phosphorylated
10 compound. This compound (20 mg) was dissolved in a mixture of methanol (2 mL) and water (1 mL) and hydrogenated over a 10 % Pd-on-C catalyst (10 mg) at rt for 62 h. The catalyst was removed by filtration and the methanol was evaporated. The residue was treated with ammonium bicarbonate solution and
15 subsequently frozen and lyophilized. Purification, if necessary, was by the same procedure as in method A.
(N-Methanocarba-2'-deoxyadenosine-3',5'-bis(diammonium phosphate) (4a) [(IR,2S,4S,5S)-1-[(phosphato)methyl]-4-(6-aminopurin-9-yl) bicyclo [3.1.0.]-hexane-2-phosphate
20 **tetraammonium salt]**
Starting from 16 mg (0.06 mmol) of ($\overline{N}$)-methanocarba-2'deoxyadenosine and following the general phosphorylation procedure A we obtained 1.8 mg (0.0037 mmol, 5.5 % yield) of the desired compound.

25 ^{1}H-NMR (D_2O) ∂ 0.90 (1H, m, CH_2-6), 1.10 (1H, m CH_26'), 1.82 (1H, m, CH-5), 1.91 (1H, m, CH_2-3') 2.23 (1H, m, CH_2-3'), 3.49 (1H, d, J = 11.7 Hz, CH_2-OH), 4.16 (1H, d, J = 6.9 Hz, CH_2-2'), 8.39 (1H, s, H-2), 8.54 (1H, s, H-8).

^{31}P-NMR (D_2O) ∂ 0.43 (s, 5'P); -0.19 (s, 3'P).

(*N*)-Methanocarba-N[6]-methyl-2'deoxyadenosine-3',5'-
bis(diammonium phosphate) (4b)

(*1R,2S,4S,5S*)-1-[(phosphato)methyl]-4-(6-methylaminopurin-9-
yl) bicyclo [3.1.0.]-hexane-2-phosphate tetraammonium salt]

13.5 mg (0.0170 mmol) of compound 18 was converted to the
corresponding phosphoric acid analog using hydrogenation
following the general procedure B. Purification was performed
on an ion-exchange column packed with Sephadex-DEAE A-25
resin, linear gradient (0.01 to 0.5 M) of 0.5 M ammonium
bicarbonate was applied as the elan to give 3.0 mg (0.0060
mmol, 35.3 % yield) of the desired compound.

^{1}H-NMR (D$_2$P) ∂ 0.93-0.98 (1H, m, CH$_2$-6'), 1.17 (1H, m, CH2-6'),
1.86-1.88 (1H, m, CH5'), 1.94-1.98 (1H, m, CH$_2$-3'), 2.23-2.31
(1H, m, CH$_2$-3'), 3.09 (3H, bs, N[6]-CH$_3$), 3.61-3.64 (1H, m,
CH$_2$OH), 4.51-4.55 (1H, m, CH$_2$OH), 5.01-5.03 (1H, m, CH-4'),
5.19-5.21 (1H, m, CH-2'), 8.22 (1H, s, H-2), 8.51 (1H, s, H-
8). 31P-NMR (D$_2$O) ∂ 1.26, 1.92 (2s, 3'-P, 5'-P).

(*N*)-Methanocarba-N[6]-methyl-2-chloro-2'-deoxyadenosine-3',5'-
bis(diammonium phosphate) (4c)

[(*1R,2S,4S,5S*)-1[(phosphato)methyl]-4-(2-chloro-6-aminopurin-
9-yl) bicyclo [3.1.0]-hexane-2-phosphate tetraammonium salt]

The nucleoside, compound 23, reacted with tetrabenzyl
pyrophosphate, as in Method B, followed by an alternative
deprotection procedure. Starting from 10 mg (0.0323 mmol) of
(*N*)-methanorcarba-N[6]-methyl-2-chloro-2'-deoxyadenosine and
following the general phosphorylation procedure (Method B) we
obtained 9.5 mg (0.0114 mmol, 35.3 % yield) of the desired

compound,   (N)-methanocarba-N^6-methyl-2-chloro-2'-deoxyadenosine-3',5'-bis(dibenzyl phosphate).

1H-NMR (CDCl$_3$) ∂ 0.75-0.81 (H, m, CH$_2$-6'), 103-1.08 (1H, m, CH$_2$-6'), 1.49-1.51 (1H, m, CH-5'), 1.84-1.94 (1H, m, CH$_2$-3'), 1.99-
2.10 (1H, m, CH$_2$-3'), 3.12 (3H, bs, N^6-CH$_3$), 4.11-4.20 (1H, m, CH$_2$OH), 4.50-4.55 (H, m, CH$_2$OH), 4.90-4.98, (8H, m, - OCH$_2$), 4.99-5.01 (1H, m, CH-4'), 5.23-5.30 (1H, m, CH-2'), 5.90 (1H, BS, NH), 7.20-7.29 (20H, m, C$_6$H$_5$), 7.82 (1H, s, H-8)

^{31}P-NMR (D$_2$O) ∂ -0.58 (s,5'P); -1.06 (s,3'P).

MS(CI-NH$_3$) (M+1) 830 HRMS (FAB-) (M+Cs) Calcd. 962.1252; Found 962, 1252

      9.5 mg (0.0114 mmol) of the tetrabenzyl-protected intermediate added to dry CH$_2$Cl$_2$ (1.0 mL) was cooled to -78 °C under argon and treated with 100 µL of boron trichloride solution (1M in CH$_2$Cl$_2$) and 100 µL of anisole. The reaction mixture was stirred for 12 hr at 0 °C to rt and extracted with triethylamine solution. Purification was performed on an ion-exchange column packed with Sephadex-DEAE A-25 resin, linear gradient (0.01 to 0.5 M) of 0.5 M ammonium bicarbonate was applied as the eluent to give 0.4 mg (0.0007 mmol, 6.52 yield) of the desired compound 4c.

^{1}H-NMR (D$_2$O) ∂ 0.91-0.96 (1H, m, CH$_2$-6'), 1.12-1.16 (1H, m, CH$_2$-6'), 1.80-1.84 (1H, m, CH-5'), 1.85-1.98 (1H, m, CH$_2$-3'), 2.20-2.50 (1H, m, CH$_2$-3'), 3.08 (3H, bs, N^6-CH$_3$), 3-57-3.60 (1H, m, CH$_2$OH), 4.52-4.67 (1H, m, CH$_2$OH), 4.94-4.96 (1H, m, CH-4'), 5.18-5.21 (1H, m, CH-2'), 8.52 (1H, s,H-8)

^{31}P-NMR (D$_2$O) ∂ 1.82, 2.52 (2s, 3'-P, 5'P)

(S)-Methanocarba-2',   deoxyadeonosine-3',5'-bis(diammonium
phosphate   (5)   [(1S,3S,4R,5S)-4-[(phosphato)methyl]-1-(6-
aminopurin-9-yl)   bicyclo   [3.1.0]-hexane-3-phosphate
tetraammonium salt]

5   Starting from 16 mg (0.06 mmol) of (S)-methanocarba-
2'deoxyadenosine and following the general phosphorylation
procedure A, we obtained 2.1 mg (0.0043 mmol, 7.55 yield) of
the desired compound 5.

^{1}H-NMR (D$_2$O) ∂ 1.36 (1H, m, CH$_2$-6'), 1.53 (1H, t, J = 4.8 Hz,
10   CH$_2$-6'), 2.05 (1H, m, CH$_2$-5'), 2.30 (1H, m, CH-4'), 2.46 (2H,
m, CH$_2$-2'), 3.97 (2H, m, CH$_2$OH), 4.45 (1H, d, J = 6.6Hz, CH-
3'), 8.16 (1H, s, H-2), 8.30 (1H, s, H-8). ^{31}P-NMR (D$_2$O) ∂ 0.85
(bs, 5'P); 0.31 (bs, 3'P).

[(1S,3S,4R,5S)-1-[(Hydroxy)methyl]-2-hydroxy-4-(6-
15   methylaminopurin-9-1yl) bicyclo [3.1.0]-hexane (17b)

The Dimroth rearrangement (Scheme 2) was carried out on (N)-
methanocarba-2'-deoxyadenosine.   Specifically, the   (N)-
methanorcarba-2'-deoxyadenosine (17a, 50.0 mg, 0.191 mmol) was
heated at 40° C with methyl iodide (71.5 µL, 1.15 mmol) in dry
20   DMF (2.0 mL) for 48 h.   The solvent was evaporated under
reduced pressure, and the residue was heated at 90 °C with
ammonium hydroxide (4.0 mL) for 4 h.   The water was
evaporated, and the residue was purified by pTLC using MeOH;
CHCl$_3$ (1:9) to afford compound 17b as a colorless solid (40
25   mg, 0.15 mmol, 76%).

^{1}H-NMR (CD$_3$OD) ∂ 0.77.-0.81 (1H, m, CH$_2$-6'), 1.03-1.07 (1H, m,
CH$_2$-6'), 1.68-1.72 (1H, m, CH-5'), 1.79-1.89 (1H, m, CH$_2$-3'),
2.00-2.07 (1H, m, CH$_2$-3'), 3.12 (3H, bs, N^6-CH$_3$), 3-33 (1H, d,
J = CH$_2$OH), 4.29 (1H, d, J = 11.7 Hz, CH$_2$OH), 4.89-4.92 (1H, m,

CH-4'), 5.02 (1H, d, J = 6.9 Hz, CH-2'), 8.24 (1H, s,H-2), 8.49 (1H, s, H-8).

MS(CI-NH$_3$) : 276 (M+1) 830 HRMS(FAB-) (M+Cs) Calcd. 275.1382; Found 275.1389.

5   **(N)-Methanocarba-N^6-methyl-2'-deoxyadenosine-3',5'-bis(dibenzylphosphate) (18)**

[(1S,2S,42,5S)-1-[(dibenzylphosphato)methyl]-4-(6-methylaminopurin-9-yl)   bicyclo   [3.1.0]-hexane-2-dibenzylphosphate]

10   Starting from 20.0 mg (0.0726 mmol) of N-methanorcarba-N^6-methyl-2'-deoxyadenosine **17b** and following the general phosphorylation procedure (Method B we obtained 13.5 mg (0.0170 mmol, 23.4 % yield) of the desired protected intermediate, **18** as shown in Scheme 2.

15   ^{1}H-NMR (CDCl$_3$) ∂ 0.73-0.78 (1H, m, CH$_2$-6'), 0.94-.0.98 (1H, m, CH$_2$-6'), 1.53-1.54 (1H, m, CH-5'),. 1.81-1.91 (1H, m, CH$_2$-3'), 2.05-2.13 (1H, m, CH$_2$-3'), 3.15 (3H, bs, N^6-CH$_3$), 3-70-3.83 (1H, m, CH$_2$OP), 4.49-4.55 (1H, m, CH$_2$OP), 4.89-5.00 (8H, m, OCH-$_2$), 5.02-5.06 (1H, m, CH-4'), 5.27-5.32 (1H, m, CH-2'), 20   5.86 (1H, bs, NH), 7.21-7.23 (20H, m, C$_6$H$_5$), 7.86 (1H, s,H-2), 8.31 (1H, s,H-8).^{31}P-NMR (D$_2$O) ∂ -0.56, -1.05 (2s, 3'-P, 5'P) HRMS (FAB-) (M-Cs) Calcd. 928.1641; Found 928.1700.

[(1S,2S,42,5S)-1-[(Benzyloxy)methyl]-2-benzyloxy-4-(2-6-dichloropurin-9-yl) bicyclo [3.1.0]-hexane (21)

25   To an ice cold solution of triphenylphosphine (278 mg, 1.06 mmol) in dry THF (2 mL) was added diethylazadicarboxylate (170 µL, 1.06 mmol) dropwise under a nitrogen atmosphere, and the mixture was stirred for 20 min until the solution turned red orange (Scheme 3). This mixture was added dropwise to a cold

stirred mixture of the starting alcohol (135 mg, 0.417 mmol) and 2.6-dichloropurine (157 mg, 0.883 mmol) under a nitrogen atmosphere. The reaction mixture was stirred in an ice bath for 30 min and then allowed to warm to room temperature, and

5   stirring continued for 12 h. Solvent was removed by nitrogen purge, and the residue was purified by pTLC using EtOAc : petroleum ether (1 : 1) to afford a thick liquid (132 mg, 0.263 mmol, 64%).

^{1}H NMR: (CD$_3$OD) δ 0.85 (m, 1H), 1.13 (m, 1H), 1.59 (m, 1H),

10   1.68 (m, 1H), 2.06 (m, 1H), 3.17 (d, J = 10.8 Hz, 1H), 4.11-4.57 (m, 5H), 5.20 (d, J = 6.9 Hz, 1H), 6.6 (bs, 1H), 7.23-7.37 (m, 10H), 8.98 (s, 1H).

MS : (EI) 494 (M+).

[(1R,2S,4S,5S)-1-[(Benzyloxy)methyl]-2-benzyloxy-4-(2-chloro-

15   **6-methylaminopurin-9-yl) bicyclo [3.1.0]-hexane (22)**

Compound 21 (100 mg, 0.202 mmol) was dissolved in methylamine in methanol (30 % solution, 3mL) and was stirred at rt for 12 h under a nitrogen atmosphere. The solvent was evaporated, and the crude product was purified by pTLC using EtOAc :

20   petroleum ether (6 : 4) to afford 22 as a light yellow solid (86 mg, 0.176 mmol, 88 %).

^{1}H NMR: (CD$_3$OD) δ 0.70 (m, 1H), 1.06 (m, 1H), 1.50 (m, 1H), 1.76 (m, 1H), 1.96 (m, 1H), 3.01 (s, 3H), 3.08 (m, 2H), 4.03 (m, 4H), 4.45 (bs, 1H), 5.02 (bs, 1H), 8.38 (s, 1H).

25   MS : (Cl) : 490 (M+1).

[(1R,2S,42,5S)-1-[(Hydroxy)methyl]-2-hydroxy-4-(2-chloro-6-
methylaminopurin-9-yl) bicyclo [3.1.0]-hexane (23)

Compound 22 (40 mg 0.0816 mmol) was dissolved in dry CH$_2$Cl$_2$ (1.0 mL), and hydrogenated using BCl$_3$ (1M in CH$_2$Cl$_2$, 175 μL)

30   for 50 min at -78 °C under argon. The solvent was evaporated,

and the crude product was purified by pTLC using $CHCl_3$: MeOH

(10 : 1) to afford **23** as a light yellow solid (10.0 mg, 0.0323

mmol, 39.6 %).

^{1}H NMR: (CD_3OD) ∂ 0.77-0.81 (1H, m, CH_2-6'), 1.02-1.05 (1H, m,

CH_2-6'), 1.65-1.68 (1H, m, CH-5'), 1.78-1.91 (1H, m, CH_2-3'),

1.99-2.07 (1H, m, CH_2-3'), 3.08 (3H, bs, N^6-CH_3), 3.37 (1H, d,

J = 11.7 Hz, CH_2OH), 4.27 (1H, d, J = 11.7 Hz, CH_2OH), 4.89-

4.91 (1H, m, CH-4), 4.97 (1H, d, J = 6.8 Hz, CH-2'), 8.46 (1H,

s, H-8).

MS : ($CI-NH_3$): 310 (M+1), HRMS (FAB-): Calcd 309.0992, Found

309.0991.

Pharmacological Analyses.

$P2Y_1$ receptor promoted stimulation of inositol phosphate

formation by adenine nucleotide analogues was measured in

turkey erythrocyte membranes as previously described. The $K_{0.5}$

values were averaged from 3-8 independently determined

concentration-effect curves for each compound. Briefly, 1 mL

of washed turkey erythrocytes was incubated in inositol-free

medium (DMEM; Gibco, Gaithersburg MD) with 0.5 mCi of 2-

$[^3H]$ myo-inositol (20Ci/mmol: American Radiolabelled Chemicals,

Inc., St. Louis MO) for 18-24 h in a humidified atmosphere of

95% air/5% CO_2 at 37 °C. Erythrocyte ghosts were prepared by

rapid lysis in hypotonic buffer (5 mM sodium phosphate, pH

7.4, 5 mM $MgCI_2$, 1mM EGTA) as described. Phospholipase C

activity was measured in 25 µL of $[^3H]$ inositol-labeled ghosts

(approximately 175 µg of protein, 200-500,000 cpm/assay) in a

medium containing 424 µM $CaCl_2$, 0.91 mM $MgSO_4$, 2mM EGTA, 115 mM

KCl, 5 mM KH_2PO_4, and 10 mM Hepes pH 7.0. Assays (200 µL

final volume) contained 1 μM GTPγS and the indicated
concentrations of nucleotide analogues. Ghosts were incubated
at 30 °C for 5 min, and total [^{3}H]inositol phosphates were
quantitated by anion exchange chromatography as previously
5 described.[7,36]

Data analysis.

Agonist potencies were calculated using a four-parameter
logistic equation and the GraphPad software package (GraphPad,
San Diego, CA). EC_{50} values (mean ± standard error) represent
10 the concentration at which 50 % of the maximal effect is
achieved. Relative efficacy (%) was determined by comparison
with the effect produced by a maximal effective concentration
of 2-MeSADP in the same experiment.

Antagonist IC_{50} values (mean ± standard error) represent
15 the concentration needed to inhibit by 50 % the effect
elicited by 10 nM 2-MeSADP. The percent of maximal inhibition
is equal to 100 minus the residual fraction of stimulation at
the highest antagonist concentration.

All concentration-effect curves were repeated in at least
20 three separate experiments carried out with different membrane
preparations using duplicate or triplicate assays.

53

Table 3

Stimulation of PLC at turkey erythrocyte $P2Y_1$ receptors (agonist effect) and the inhibition of PLC stimulation elicited by 10 nM 2-MeSADP (antagonist effect), for at least
5　two separate determinations.

Compound	Agonist Effect, % of maximal increase[a]	$EC_{50}, \mu M$[a]	Antagonist Effect, % of maximal inhibition[b]	$IC_{50}, \mu M$[b] (n)
1a[c,e] (MRS 2179)	NE		99 ± 1	0.331 ± 0.059 (5)
1b[e]	NE		95 ± 1	0.206 ± 0.053
1c[e]	4	d	96 ± 2	1.85 ± 0.74
1d[e]	6 ± 2	d	94 ± 2	0.362 ± 0.119
4a	92 ± 5	0.155 ± 0.021	NE	
4b	NE		100	0.157 ± 0.060
4c	NE		100	0.0516 ± 0.0008
5	41 ± 13	13.3	34% at 100 μM	small decrease

a　Agonist potencies were calculated using a four-parameter logistic equation and the GraphPad softaware package (GraphPad, San Diego, CA). EC_{50} values (mean ± standard
10　error) represent the concentration at which 50% of the maximal effect is achieved. Relative efficacies (%) were determined by comparison with the effect produced by a maximal effective concentration of 2-MeSADP in the same experiment. Small increase refers to <10% at 100 μM.
15　b Antagonist IC_{50} values (mean ± standard error) represent the concentration needed to inhibit by 50% the effect elicited by 10 nM 2-MeSADP. The percent of maximal inhibition is equal to 100 minus the residual fraction of stimulation at the highest antagonist concentration.
20　c. 1a, MRS 2179; 4c, MRS 2279.
d　EC_{50} was not calculated for increases of　10% at 100 μM.
e　values from refs. 17, 19.
NE　no effect at 100 μM.

54

TABLE 4

Synthetic data for nucleotide derivatives, including structural verification using high resolution mass spectroscopy and purity verification using HPLC.

No	Formula	FAB $(M-H^+)$ Calcd	Found	HPLC (rt; min)[a] System A	System B	Method, Yield (%)[b]
2	$C_{10}H_{15}O_9N_5P_2$	410.0267	410.0269	3.53	10.72	B, 21.7
3b	$C_{12}H_{19}O_8N_5P_2$	422.0631	422.0664	3.41	8.21	B, 8.0
4a	$C_{12}H_{17}O_8N_5P_2$	420.0474	420.0482	3.92	7.30	A, 5.5
4b	$C_{13}H_{19}O_8N_5P_2$	434.0631	434.0622	5.91	7.83	B, 8.3
4c	$C_{13}H_{18}O_8N_5P_2Cl$	468.0241	468.0239	8.05	8.54	B, 2.3
5	$C_{12}H_{17}O_8N_5P_2$	420.0474	420.0481	4.02	6.84	A, 7.5
6	$C_{11}H_{16}O_8N_5P_2Cl$	442.0084	442.0070	6.67	6.82	A, 24.3
7b	$C_{12}H_{20}O_{12}N_5P_3$	518.0237	518.0243	4.98	12.74	A, 1.8
7c	$C_{12}H_{19}O_9N_5P_2$	438.0580	438.0580	4.63	9.36	B, 50.1
7d	$C_{12}H_{18}O_9N_5P_2Cl$	472.0201	472.0190	5.67	9.97	B, 31.3
8a	$C_{12}H_{20}O_8N_6P_2$	437.0740	437.0721	2.37	8.78	8.0
8b	$C_{12}H_{21}O_{11}N_6P_3$	517.0403	517.0404	2.42	9.23	7.2
8c	$C_{12}H_{22}O_{14}N_6P_4$	597.0066	597.0053	2.96	10.02	4.0

[a] Purity of each derivative was 95%, as determined using HPLC with two different mobile phases. System A: gradient of 0.1M TEAA/CH_3CN from 95/5 to 40/60 and System B: gradient of 5mM TBAP/ CH_3CN from 80/20 to 40/60.

[b] Phosphorylation methods: Method A refers to use of phosphorous oxychloride, and Method B refers to use of tetrabenzyl pyrophosphate/lithium diisopropylamide followed by hydrogenation. The percent yields refer to overall yield for each phosphorylation sequence. For the method of synthesis of 8 refer to Experimental Section.

Abbreviations

AIBN, 2,2'-azobisisobutyronitrile;

ATP, adenosine 5'-triphosphate;

DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene;

DCTIDS, 1,3-dichlorotetraisopropyl-1,1,3,3,-disiloxane;

DEAD, diethylazadicarboxylate;

DEAE, diethylaminoethyl;

DMAP, 4-dimethylaminopyridine;

5   DMF, dimethylformamide;

DMSO, dimethylsulfoxide;

FAB, fast atom bombardment (mass spectroscopy);

HPLC, high pressure liquid chromatography;

MS, mass spectroscopy;

10   HRMS, high resolution mass spectroscopy;

LDA, lithium diisoproylamide;

2-MeSADP, 2-methylthioadenosine-5'-diphosphate;

TBAP, tetrabutylammonium phosphate;

TEAA, triethylammoniun acetate;

15   THF, tetrahydrofuran;

All of the references cited herein, including patents,
patent applications, and publications, are hereby incorporated
in their entireties by reference.

While this invention has been described with an emphasis
20   upon preferred embodiments, it will be obvious to those of
ordinary skill in the art that variations of the preferred
embodiments may be used and that it is intended that the
invention may be practiced otherwise than as specifically
described herein. Accordingly, this invention includes all
25   modifications encompassed within the spirit and scope of the
invention as defined by the following claims.

WHAT IS CLAIMED IS:

1.   A compound having the formula A-M, wherein A is a chemically modified adenine or uracil and M is a constrained cycloalkyl group, said adenine or uracil is bonded to said constrained cycloalkyl group, and said compound binds a receptor; or a salt of said compound.

2.   The compound of claim 1, wherein said receptor is a P1 or P2 receptor.

3.   The compound of claim 2, wherein said P1 receptor is selected from the group consisting of A_1, A_{2A}, and A_3.

4.   The compound of claim 2, wherein said P2 receptor is selected from the group consisting of P2Y and P2X.

5.   The compound of claim 1, wherein said constrained cycloalkyl group includes a cyclopentyl group.

6.   The compound of claim 3, wherein said constrained cyclopentyl group is a cyclopentyl ring derivatized with a fused cyclopropane bridge.

7.   The compound of claim 1, wherein said constrained cycloalkyl group is constrained in the N- conformation.

8.   The compound of claim 1, wherein said constrained cycloalkyl group is constrained in the S- conformation.

9.   A compound selected from the group consisting of

and
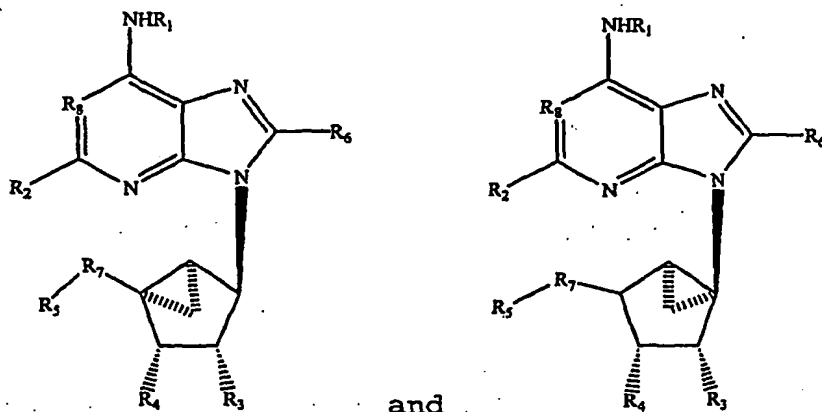
wherein

R_1 is hydrogen, alkyl, cycloalkyl, alkoxy, cycloalkoxy, aryl,
5   arylalkyl, acyl, sulfonyl, arylsulfonyl, thiazolyl or
bicyclic alkyl;

R_2 is hydrogen, halo, alkyl, aryl, arylamino, aryloxide,
alkynyl, alkenyl, thiol, cyano, or ;

R_3, R_4, and R_5, are each independently hydrogen, hydroxyl,
10   alkoxy, alkyl, alkenyl, alkynyl, aryl, acyl, alkylamino,
arylamino, phosphoryl, diphosphoryl, triphosphoryl,
phosphonyl, boronyl, thiophosphoryl, thiodiphosphoryl,
thiotriphosphoryl or vanadyl, and can be the same or
different;

15   R_6 is hydrogen, alkyl, alkenyl, alkynyl, heteroaryl or
aminoalkyl;

R_7 is methylene, dihalomethyl, carbonyl, sulfoxide;
and at least one of R_1, R_2, and R_6 is other than hydrogen;

R_8 is carbon or nitrogen;

20   or a salt of said compound.

58

10. The compound of claim 9, wherein
R_1 is alkyl, cycloalkyl, alkoxy, aryl, arylalkyl,
bicycloalkyl, or sulfonyl.

11. The compound of claim 9, wherein
5   R_1 is methyl, cyclopentyl, cyclohexyl, phenyl, R-
phenylisopropyl, benzyl, or phenylethyl;

R_2 is chloro;

and R_6 is C_1-C_6 alkylamino, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6
alkynyl.

10   12. The compound of claim 9 or 10, wherein R_1 is further
substituted with a member selected from the group consisting
of hydroxyl, halo, sulfonyl, amino, cyano, alkoxyl, alkyl,
alkenyl, alkynyl, cycloalkyl, aryl, arylalkyl, sulfonamido,
carboxyl, and carboxamido.

15   13. The compound of claim 9, wherein R_1 is methyl group
and R_2 is chloro, alkylthio, or arylalkylthio.

14. The compound of claim 9, wherein R_6 is methyl and R_2
is chloro, alkylthio, arylalkylthio or hydrogen.

15. The compound of claim 9, wherein R_6 is halo and R_2 is
20   a chloro, alkylthio, arylalkylthio or hydrogen.

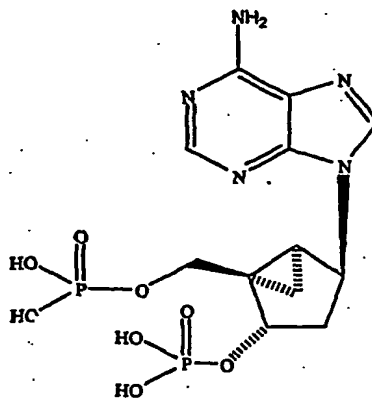16. The compound of claim 9, wherein R_2 is chloro.

17. The compound of claim 9, wherein R_1 is methyl and R_2
is chloro and R_3 is hydrogen.

18.   The compound of claim 9, wherein the compound has the formula
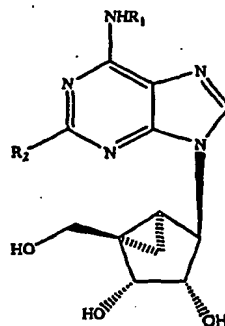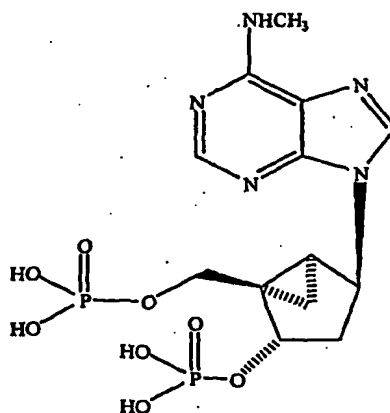

5   wherein R₁ is iodobenzyl, or cyclopentyl and
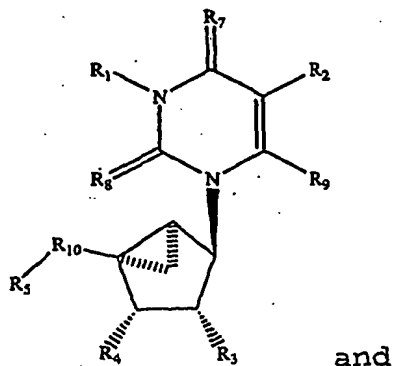
R₂ is hydrogen or chloro.

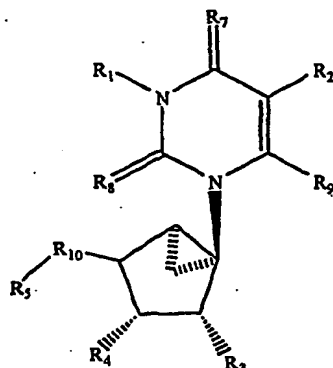19. The compound of claim 9, wherein the compound has
the formula

20. The compound of claim 9, wherein the compound has
5   the formula

21. A compound selected from the group consisting of:

and

61

wherein

R_1, R_2, R_9 is hydrogen, alkyl, alkenyl, alkynyl, aminoalkyl and

R_3, R_4, and R_5, are each independently hydrogen, hydroxyl,

5   alkoxy, alkyl, alkenyl, alkynyl, aryl, acyl, alkylamino,

arylamino, phosphoryl, phosphonyl, boronyl, or vanadyl, and

can be the same or different;

R_6 and R_7 are each independently sulfur or oxygen;

and R_{10} is methylene, dihalomethyl, carbonyl, sulfoxide;

10   or a salt of said compound.

22. The compound of claim 21, wherein R_1 is methyl.

23. A compound comprising a methanocarbocyclic analog of

a chemically modified adenosine or uradine wherein said

compound is a P2 receptor ligand; or a salt of said compound.

15   24. The compound of claim 23, wherein the compound is a

P2 receptor agonist.

25. The compound of claim 23, wherein the compound is a

P2 receptor antagonist.

26. The compound of claim 22, wherein said P2 receptor

20   is selected from the group consisting of P2Y and P2X.

27. The compound of claim 22, wherein said P2 receptor is a P2Y receptor.

28. The compound of claim 22, wherein said P2 receptor is a P2Y1 receptor.

5   29. The compound of claim 22, wherein said P2 receptor is a P2X receptor.

30. A compound comprising a methanocarbocyclic analog of a chemically modified adenosine or uradine wherein said compound is a P1 receptor ligand; or a salt of said compound.

10   31. The compound of claim 30, wherein the compound is a P1 receptor agonist.

32. The compound of claim 30, wherein the compound is a P1 receptor antagonist.

33. The compound of claim 30, wherein said P1 receptor
15   is selected from the group consisting of A_1, A_{2A}, and A_3.

34. The compound of claim 30, wherein said P1 receptor is A_1 receptor.

35. The compound of claim 30, wherein said P1 receptor is A_3 receptor.

20   36. A method of treating or preventing in a mammal a disease, state, or condition that responds to an adenosine, ATP, or UTP receptor agonist or antagonist comprising administering to the mammal a compound of any of any of claims 1-36.

25   37. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of any of claims 1-36.

33. The use of a compound of any of claims 1-36 as a medicament.

34. The use of a methanocarba analog in the manufacture of a medicament for the treatment or prevention in a mammal a
5 disease state, or condition that responds to an adenosine, ATP, UTP receptor agonist or antagonist.

35. A method for the treatment of airway diseases, cancer, cardiac arrhythmia, cardiac ischemia, epilepsy, Huntington's Disease, immunodeficient disorders, inflammatory
10 disorders, neonatal hypoxia, neurodegenerative, pain, Parkinson's Disease, renal failure, schizophrenia, sleep disorders, stroke, thrombosis, urinary incontinence, diabetes, psoriasis, septic shock, brain trauma, glaucoma, or congestive heart failure in individuals in need of such treatment
15 comprising contacting an effective quantity of a compound of any of claims 1-36.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   C07D471/04   C07D487/04   C07D239/54   C07D239/56   C07D239/58
      A61P29/00   A61P35/00   A61K31/505   A61K31/52
      //(C07D487/04,239:00,235:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   C07D   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIDDIQUI M A ET AL: "SYNTHESIS, CONFORMATIONAL ANALYSIS, AND BIOLOGICAL ACTIVITY OF A RIGID CARBOCYCLIC ANALOGUE OF 2'-DEOXYARISTEROMYCIN BUILT ON A BICYCLOU3.1.OHEXANE TEMPLATE" NUCLEOSIDES & NUCLEOTIDES,US,MARCEL DEKKER, INC, vol. 15, no. 1/03, 1996, pages 235-250, XP000615548 ISSN: 0732-8311 cpds 1-4,14 --- -/--	9-22,37, 38

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the International filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the International filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 May 2001	15/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Fritz, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KATAGIRI N ET AL: "The first synthesis of a 2',3'-methano carbocyclic nucleoside" TETRAHEDRON LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 40, no. 51, 17 December 1999 (1999-12-17), pages 9069-9072, XP004184808 ISSN: 0040-4039 cpds. B-D,13	9-22,37, 38
X	DYATKINA N ET AL: "Modified triphosphates of carbocyclic nucleoside analogues: synthesis, stability towards alkaline phosphatase and substrate properties for some DNA polymerases" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS,GB,OXFORD, vol. 6, no. 22, 19 November 1996 (1996-11-19), pages 2639-2642, XP004135866 ISSN: 0960-894X Cpds. 6-8	9-20,37, 38
X	WO 95 08541 A (US HEALTH) 30 March 1995 (1995-03-30) cpds. 5,13-16,7-9,18-22	9-22,37, 38
X	WO 95 03304 A (MERRELL DOW PHARMA) 2 February 1995 (1995-02-02) the whole document	9-22,37, 38
X	EP 0 577 558 A (CIBA GEIGY AG) 5 January 1994 (1994-01-05) compounds (I), (Ia) claims	9-22,37, 38
X	US 5 840 728 A (RODRIGUEZ JUAN B ET AL) 24 November 1998 (1998-11-24) examples; claims	9-22,37, 38
X	WO 98 05662 A (US HEALTH) 12 February 1998 (1998-02-12) Schemes; examples	9-20,37, 38
X	US 5 629 454 A (RODRIGUEZ JUAN B ET AL) 13 May 1997 (1997-05-13) examples	9-22,37, 38

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	LAK S J ET AL: "Use of a Cyclic Sulfite as an Epoxide Surrogate in the Regioselective Synthesis of a Carbocyclic Ring-Enlarged 4@?,1'a-Methano Oxetanocin Analogue" TETRAHEDRON LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 37, no. 14, 1 April 1996 (1996-04-01), pages 2353-2356, XP004029940 ISSN: 0040-4039 Cpds. 2,4,6,17-18	9-22,37, 38
X	V E MARQUEZ ET AL: "Nucleosides with a Twist. Can Fixed Forms of Sugar Ring Pucker Influence Biological Activity in Nucleosides and Oligonucleotides ?" JOURNAL OF MEDICINAL CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. WASHINGTON, vol. 39, 13 September 1996 (1996-09-13), pages 3739-3747, XP002094300 ISSN: 0022-2623 cpds. 7-13	9-22,37, 38
X	V. E. MARQUEZ ET AL.: "HIV-1 Reverse Transcriptase Can Discriminate between Two Conformationally Locked Carbocyclic AZT Triphosphate Analogues" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 120, 8 October 1997 (1997-10-08) - 17 March 1998 (1998-03-17), pages 2780-2789, XP000996607 Washington Cpds. 1-3,6-11	21,22, 37,38
X	A. EZZITOUNI ET AL.: "Conformationally locked carbocyclic nucleosides built on a bicyclo'3.1.0!hexane template with a fixed Southern conformation. Synthesis and antiviral activity" JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS, vol. 1, 24 June 1996 (1996-06-24) - 1997, pages 1073-1078, XP000996608 Letchworth, GB cpds. 23-24,28	9-22,37, 38
	—/—	

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	V. E. MARQUEZ ET AL.: "Synthesis of Conformationally Restricted Carbocyclic Nucleosides: The Role of the O(4')-Atom in the Key Hydration Step of Adenosine Deaminase" HELVETICA CHIMICA ACTA, vol. 82, 23 August 1999 (1999-08-23), pages 2119-2129, XP000099613 Basel, CH cpds. 8-10,20	9-20,37, 38
X	K. J. SHIN ET AL.: "Construction of the Bicyclo'3.1.0!hexane Template of a Conformationally Locked Carbocyclic Adenosine via an Olefin Keto-Carbene Cycloaddition" JOURNAL OF ORGANIC CHEMISTRY, vol. 65, 15 November 1999 (1999-11-15) - 14 March 2000 (2000-03-14), pages 2172-2178, XP000996614 Washington, USA cpds. 1-4	9-20,37, 38
X	H. R. MOON: "Synthesis of Cyclopropyl-Fused Carbocyclic Nucleosides via the Regioselective Opening of Cyclic Sulfites" JOURNAL OF ORGANIC CHEMISTRY, vol. 64, 4 January 1999 (1999-01-04) - 6 June 1999 (1999-06-06), pages 4733-4741, XP000996615 Washington, USA cpds. 18-20,3a,3c	9-22,37, 38
X	A. EZZITOUNI: "(1S,2R)-'(Benzyloxy)methyl!cyclopent-3-en ol. A versatile Synthon for the Preparation of 4',1'a-Methano- and 1',1'a-Methanocarbocyclic Nucleosides" JOURNAL OF ORGANIC CHEMISTRY, vol. 62, 13 November 1996 (1996-11-13) - 1997, pages 4870-4873, XP000996616 Washington, USA cpds.1,2,16	9-22,37, 38

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	K.-H. ALTMANN ET AL.: "4',6'-Methano-Carbocyclic Thymidine: A Conformationally Constrained Building Block for Oligonucleotides" TETRAHEDRON LETTERS, vol. 35, no. 15, 24 January 1994 (1994-01-24) – 14 February 1997 (1997-02-14), pages 2331-2334, XP000996351 AMSTERDAM cpd. 5	21,22, 37,38
X	A. EZZITOUNI ET AL.: "A Simple Approach to 1',1'a-Methano Carbocyclic Thymidine" J. CHEM. SOCIETY, CHEM. COMM., 28 March 1995 (1995-03-28), pages 1345-1346, XP000996358 Letchworth, GB Cpds. 1,2	21,22, 37-39
X	F. THEIL ET AL.: "Chemoenzymatic synthesis of carbocyclic nucleoside analogues with bicyclo'3.1.0!hexyl residues" J. CHEM. SOC., PERKIN TRANS., vol. 1, 2 August 1995 (1995-08-02) – 15 September 1995 (1995-09-15), pages 255-258, XP000996355 Letchworth, GB cpd. 8	9-20,37, 38
X	J. B. RODRIGUEZ ET AL.: "Synthesis of Cyclopropane-fused Dideoxycarbocyclic Nucleosides Structurally Related to Neoplanocin C" TETRAHEDRON LETTERS, vol. 34, no. 9, 8 – 30 July 1993, pages 6233-6236, XP000996359 Amsterdam, NL cpds. 5,7-8,10,18-20	9-22,37, 38
X	V. E. MARQUEZ ET AL.: "Conformational Analysis of Nucleosides Constructed on a Bicyclo'3.1.0!hexane Template. Structure-antiviral Activity Analysis for the Northern and Southern Hemispheres of the Preudorotational Cycle " NUCLEOSIDES & NUCLEOTIDES, vol. 16, no. 7-9, 1997, pages 1431-1434, XP000996269 cpds. 3,7	9-20, 37-39

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	V. E. MARQUEZ: "Conformationally restricted Nucleosides. The raection of adenosine deaminase with substrates built on a bicylco'3.2.1!hexane template " NUCLEOSIDES & NUCLEOTIDES, vol. 18, no. 4-5, 1991, pages 521-530, XP000996270 Cpds. 1,4-6	9-20, 37-39
X	L. S. JEONG ET AL.: "Synthesis and Anti-HIV activity of carbocyclic ring-enlarged 4',1'a-methano oxetanocin analogues" NUCLEOSIDES & NUCLEOTIDES, vol. 16, no. 7-9, 1997, pages 1059-1062, XP000996284 Cpds. 12-14,16-22	9-22,37, 38
X	K.-H. ALTMANN ET AL.: "1',6'-Methano Carbocyclic Thymidine: Synthesis, X-ray Crystal Structure, and Effect on Nucleic Acid Duplex Stability" TETRAHEDRON LETTERS, vol. 35, no. 41, 1994, pages 7625-7628, XP000996525 compounds 1,2	21,22, 37,38
X	J. B. RODRIGUEZ ET AL.: "Conformationally Locked Nucleoside Analogues. Synthesis of Dideocarbocyclic Nucleoside Analogues Structurally Related to Neplanocin C" J. MED. CHEM., vol. 37, 1 June 1994 (1994-06-01), pages 3389-3399, XP000996507 compounds 3,7,9,12,23,25,26,28,29,30,32,33	9-22,37, 38
X	US 4 954 504 A (CHEN JEN ET AL) 4 September 1990 (1990-09-04) abstract Compounds (I) column 1, line 20 - line 24	9-20, 37-39
X	US 5 063 233 A (CHEN JEN ET AL) 5 November 1991 (1991-11-05) Compounds (I) abstract column 1, line 21 - line 26	9-20, 37-39

-/---

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	JACOBSON K A ET AL: "ADENOSINE RECEPTORS: PHARMACOLOGY, STRUCTURE-ACTIVITY RELATIONSHIPS, AND THERAPEUTIC POTENTIAL" JOURNAL OF MEDICINAL CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. WASHINGTON, vol. 35, no. 3, 7 February 1992 (1992-02-07), pages 407-422, XP002038897 ISSN: 0022-2623 cited in the application the whole document —————	39

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

Continuation of Box I.1

Although claims 36, 40 are directed to a method of treatment of the
human/animal body, the search has been carried out and based on the
alleged effects of the compound/composition.are directed to a diagnostic
method practised on the human/animal body, the search has been carried
out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.:   1-8,23-35

Present claims 1-8,23-35 relate to an extremely large number of possible
compounds. In fact, the expression "chemically modified" contains so many
possible permutations of the vbasic chemical structure that a lack of
clarity (and/or conciseness) within the meaning of Article 6 PCT arises
to such an extent as to render a meaningful search of the claims
impossible.
Consequently, the search has been carried out for those parts of the
application which do appear to be clear (and/or concise), namely claims
9-22 and 37-39 which are the only claims being supported by the
descirption.

The applicant's attention is drawn to the fact that claims, or parts of
claims, relating to inventions in respect of which no international
search report has been established need not be the subject of an
international preliminary examination (Rule 66.1(e) PCT). The applicant
is advised that the EPO policy when acting as an International
Preliminary Examining Authority is normally not to carry out a
preliminary examination on matter which has not been searched. This is
the case irrespective of whether or not the claims are amended following
receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9508541	A	30-03-1995	AT	160340 T	15-12-1997
			AU	677441 B	24-04-1997
			AU	7842094 A	10-04-1995
			CA	2172534 A	30-03-1995
			DE	69406943 D	02-01-1998
			DE	69406943 T	07-05-1998
			DK	720604 T	27-07-1998
			EP	0720604 A	10-07-1996
			ES	2111960 T	16-03-1998
			GR	3026166 T	29-05-1998
			JP	9502986 T	25-03-1997
			US	5629454 A	13-05-1997
			US	5869666 A	09-02-1999
WO 9503304	A	02-02-1995	AT	176668 T	15-02-1999
			AU	683287 B	06-11-1997
			AU	7316794 A	20-02-1995
			CA	2166692 A	02-02-1995
			DE	69416518 D	25-03-1999
			DE	69416518 T	01-07-1999
			DK	710239 T	20-09-1999
			EP	0710239 A	08-05-1996
			ES	2132419 T	16-08-1999
			GR	3029454 T	28-05-1999
			JP	9500643 T	21-01-1997
			ZA	9405246 A	20-03-1995
EP 0577558	A	05-01-1994	CA	2099712 A	02-01-1994
			JP	6100562 A	12-04-1994
			MX	9303965 A	29-04-1994
			US	5461152 A	24-10-1995
			US	5610300 A	11-03-1997
			US	5700920 A	23-12-1997
US 5840728	A	24-11-1998	NONE		
WO 9805662	A	12-02-1998	AU	6766496 A	25-02-1998
US 5629454	A	13-05-1997	US	5869666 A	09-02-1999
			AT	160340 T	15-12-1997
			AU	677441 B	24-04-1997
			AU	7842094 A	10-04-1995
			CA	2172534 A	30-03-1995
			DE	69406943 D	02-01-1998
			DE	69406943 T	07-05-1998
			DK	720604 T	27-07-1998
			EP	0720604 A	10-07-1996
			ES	2111960 T	16-03-1998
			GR	3026166 T	29-05-1998
			JP	9502986 T	25-03-1997
			WO	9508541 A	30-03-1995
US 4954504	A	04-09-1990	US	5063233 A	05-11-1991
			AU	608258 B	28-03-1991
			AU	8119387 A	19-05-1988
			CA	1288431 A	03-09-1991
			DD	273833 A	29-11-1989
			DK	596087 A	15-05-1988

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 4954504	A		EP	0267878	A	18-05-1988
			FI	874982	A	15-05-1988
			HU	45057	A,B	30-05-1988
			JP	63135384	A	07-06-1988
			NO	874749	A,B,	16-05-1988
			NZ	222527	A	28-08-1990
			PH	25044	A	28-01-1991
			PT	86120	A,B	01-12-1987
			ZA	8708527	A	16-05-1988
US 5063233	A	05-11-1991	US	4954504	A	04-09-1990
			AU	608258	B	28-03-1991
			AU	8119387	A	19-05-1988
			CA	1288431	A	03-09-1991
			DD	273833	A	29-11-1989
			DK	596087	A	15-05-1988
			EP	0267878	A	18-05-1988
			FI	874982	A	15-05-1988
			HU	45057	A,B	30-05-1988
			JP	63135384	A	07-06-1988
			NO	874749	A,B,	16-05-1988
			NZ	222527	A	28-08-1990
			PH	25044	A	28-01-1991
			PT	86120	A,B	01-12-1987
			ZA	8708527	A	16-05-1988